THE ROLE OF MIRROR-VISUAL FEEDBACK IN MODULATING BILATERAL CORTICOSPINAL EXCITABILITY AND BILATERAL PERFORMANCE GAINS FOLLOWING UNILATERAL TRAINING IN YOUNGER AND OLDER ADULTS.

Paola Reissig

Submitted in fulfilment of the requirements for the Degree of Doctor of Philosophy (Psychology) in the Faculty of Health, Psychology, School of Medicine

University of Tasmania, August 2015

DECLARATION

I, Paola Reissig, can confirm, to the best of my knowledge and belief, that:

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ACKNOWLEDGEMENTS

Firstly, I would like to sincerely thank my primary supervisor, Dr Mark Hinder. Mark, without your unwavering support, advice, thoughtful input, practical assistance, patience and time dedicated, the completion of my PhD thesis would not have been possible. Thank you for welcoming me not only to Tasmania and to UTAS but also into your life, and I know that our friendship will continue long past the submission of my PhD.

I would also like to thank my two additional supervisors, Professor Jeffery Summers and Dr Mike Garry, for their insightful suggestions and feedback, and for always taking time to answer my questions at all stages of my PhD.

To all those who agreed to participate as a guinea pig in one or more of my experiments, thank you so very much for the willingness to sacrifice your time for often little more than some tea and biscuits in return.

A special thanks goes out to my colleague and friend Rohan Puri. It is no exaggeration to say that I would not have seen this through without your open ear, kindness, patience, encouragement, and most of all your belief in my ability to finish this PhD thesis. You are truly an exceptional person and I am so glad that you have accompanied me on this journey.

A big thanks goes out to all of my friends from university, work, volleyball, and the gym. Thank you for your distractions and for your help in ensuring that I did not lose my balance completely during the last three years. Finally, to my family and friends back home in Germany, thank you for always believing in me and supporting me from afar.

GENERAL ABSTRACT

The aim of the research described in this study is to improve the understanding of how visual feedback can be employed to modulate corticospinal excitability and performance gains in younger and older adults. Adopting the clinical approach of mirror training, it was my interest to further elucidate the effects of mirror-visual feedback on neurophysiological and behavioural changes during a motor training when compared to more standard visual feedback (i.e., focussing on the hand executing the motor task). Such knowledge is necessary to further expand the use of this promising technique to treat various neurological disorders across the lifespan.

The thesis consists of six chapters. The first chapter outlines the motivation for the conducted research, while the last chapter provides the reader with an overall discussion of the results found in chapters 3 - 6. Chapter 2 reviews current literature about theories of mirror training and the phenomenon of cross-limb transfer. It furthermore familiarises the reader with the mechanisms underlying the techniques used for the majority of the present research. Moreover, the background provided in this chapter provides the conceptualisation and rationale for the current research project. Accordingly, the chapter is written on the basis of published research *at the commencement of the present thesis*. Relevant research that has been published during the last three years (i.e., during the period that the present thesis has been undertaken) will be discussed in the appropriate experimental chapters (3-5), and in the overall discussion (chapter 6).

Chapter 3 is a report of a study that investigated the degree to which mirror-visual feedback of a moving limb was able to elicit specific neurophysiological changes in the ipsilateral hemisphere when compared to more standard visual feedback

conditions in younger and older adults. Unlike previously reported, it was found that mirror-visual feedback, irrespective of age, did not lead to more pronounced changes in ipsilateral corticospinal excitability or intracortical inhibition compared to when the visual focus was on the active or passive limb. Based on these results, it was concluded that enhanced corticospinal excitability increases in the hemisphere ipsilateral to a moving limb cannot be considered the primary mechanism underlying mirror-visual feedback-related behavioural changes.

Chapter 4 reports the results of a study that aimed to consolidate previous findings of mirror-visual feedback-induced augmented cross-limb transfer in younger adults in a complex motor learning task and to further investigate the underlying mechanism(s) of such mirror-specific behavioural changes. Unlike in previous studies, the results did not demonstrate a beneficial effect of mirror-visual feedback on cross-limb transfer. Moreover, and contrary to previously proposed, the findings suggest mirror-visual feedback-induced cross-limb transfer to most likely occur due to a combination of mechanisms of “traditional” motor transfer and action observation.

Chapter 5 reports the results of a study that addressed cross-limb transfer within the ageing population, with the aim to investigate the degree to which mirror-visual feedback is able to augment cross-limb transfer within a simple ballistic motor task in general and in older adults more specifically. Even though the results did not show either behavioural or neurophysiological effects specific to the provision of mirror-visual feedback, it was shown that younger and older adults were both able to demonstrate similar amounts of cross-limb transfer. Interestingly, cross-limb transfer was associated with different parameters in the two age groups. While transfer in the younger adults was found to be predominantly associated with the performance

gains in the trained hand, it was the amount of mirror muscle activity in the inactive hand exhibited during learning that was related to subsequent transfer in the older adults. The results thus demonstrate a preserved ability of cross-limb transfer in older adults, and further suggest bilateral activation, often reported within the context of unilateral motor tasks in the ageing population, to be a compensatory and helpful mechanism with regards to transfer processes.

LIST OF PUBLICATIONS

Chapter 3

Reissig, P., Garry, M. I., Summers, J. J., & Hinder, M. R. (2014). Visual feedback-related changes in ipsilateral cortical excitability during unimanual movement: implications for mirror therapy. *Neuropsychological Rehabilitation, 24*(6), 936-957.

Chapter 4

Reissig, P, Puri, R., Garry, M. I., Summers, J. J., & Hinder, M. R. (2015). The influence of mirror-visual feedback on training-induced motor performance gains in the untrained hand. *(PLOS ONE). Scheduled publication date: 30th of October 2015.*

Chapter 5

Reissig, P., Stöckel, T., Garry, M. I., Summers, J. J., & Hinder, M. R. (2015). The role of mirror visual feedback and mirror muscle activity on cross-limb adaptations in young and older adults. *Under review (Frontiers in Aging Neuroscience).*

LIST OF CONFERENCES

1. **Reissig, P.**, Stöckel, T., Garry, M. I., Summers, J. J. & Hinder, M. R. (February 2015). The role of mirror-visual feedback and mirror muscle activity on cross-limb adaptations in younger and older adults. (Oral presentation within the “Young Investigator Award Session” at the Sensorimotor Control Meeting, Brisbane, Australia)

2. **Reissig, P.**, Garry, M. I. & Summers, J. J., Hinder, M. R. (November 2014). The role of visual feedback in mediating adaptations in the ipsilateral cortex during unilateral motor training: implications for cross-limb transfer across the lifespan. (Poster presented at the Society for Neuroscience meeting, Washington D.C., USA)

3. **Reissig, P.**, Hinder, M. R., Garry, M. I. & Summers, J. J. (July 2013). The role of visual feedback in modifying cortical excitability during motor actions: Investigating the neural underpinnings of mirror therapy. (Poster presented at the Australasian Brain Stimulation Meeting, Melbourne, Australia).

4. Summers, J. J., Hinder, M. R., **Reissig, P.** & Goss, E. L. (July 2013). Neural correlates of age-related changes in motor function: the role of cortical excitability, inhibitory mechanisms and premotor cortex function. (Oral presentation of the Human Motor Control Laboratory at the Australasian Brain Stimulation Meeting, Melbourne, Australia)

5. **Reissig, P.**, Hinder, M. R., Garry, M. I. & Summers, J. J. (July 2013). The role of visual feedback in young and older people. (Poster presented at the Tasmanian Health Science HDR student conference, Hobart, Australia).

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Chronic regional pain syndrome: CRPS

Conditioning stimulus: CS

Confidence Interval: CI

Corticospinal excitability: CSE

Cross-limb transfer: CLT

D-wave: direct wave

Electroencephalography: EEG

Electromyography: EMG

Functional magnetic resonance imaging: fMRI

Gamma amino-butyric acid: GABA

Interhemispheric inhibition: IHI

Interstimulus interval: ISI

Intracortical facilitation: ICF

I-wave: indirect wave

Long interval intracortical inhibition: LICI

Mirror therapy: MT

Mirror-visual feedback: MVF

Motor evoked potential: MEP

Non-invasive brain stimulation: NIBS

Premotor cortex (dorsal): PMd

Premotor cortex (ventral): PMv

Primary motor cortex: M1

Positron emission tomography: PET

Resting motor threshold: rMT

Short interval intracortical inhibition: SICI

Short interval intracortical facilitation: SICF

Standard deviation: SD

Standard error of the mean: SEM

Test stimulus: TS

Transcranial direct current stimulation: tDCS

Transcranial electrical stimulation: TES

Transcranial magnetic stimulation: TMS

CHAPTER 1: BACKGROUND AND MOTIVATION

1.1 General introduction

The number of people over the age of 65 is rising worldwide, resulting in a global increase in the average age of the population (Lutz, Sanderson, & Scherbov, 2008). Despite important discoveries in medicine that have contributed to this increased life expectancy, ageing is still associated with a progressive decline in a variety of cognitive, motor and other brain functions (Deary et al., 2009). Corresponding mobility deficits, due to falls or stroke, or simply due to progressive effects of ageing, have an enormous impact on the health system and impose a significant financial burden on the economy. Moreover, such deficits impact upon a person's ability to perform everyday tasks, restricting an independent lifestyle and ultimately the quality of life of the person concerned. It is clear, therefore, that designing intervention programmes that aim to assist in older adults regaining their ability to independently perform activities of daily living by minimising the acquired loss of functional capacities is of great importance in our society.

Interestingly, functional motor capacities, such as strength and dexterity, can be improved *bilaterally* by using *unilateral* training protocols; a phenomenon known as cross-limb transfer (CLT) (Scripture, Smith, & Brown, 1894; Carroll, Herbert, Munn, Lee, & Gandevia, 2006). Although CLT has been acknowledged for over a century, and rigorously demonstrated in a variety of movement tasks, it has predominantly been investigated in healthy (young) individuals (Carroll, Lee, Hsu, & Sayde, 2008; Hortobagyi et al., 2011; Perez, Tanaka, et al., 2007). The possibility of bilateral neural and behavioural adaptations following unilateral motor paradigms, however, seems even more appealing in the treatment or rehabilitation of unilateral

orthopaedic injuries (e.g. fractures) or neurological dysfunctions - which due their severity may preclude a sufficient use of bilateral training programmes - in *older* populations. Moreover, the fact that only small decrements in limb strength or function may push older adults below thresholds marking independent living capabilities, suggests that therapies which can help maintain or limit the decline of an affected limb during the period of immobilisation or immobility could be extremely important. Despite the potential promise of such unilateral training programmes utilising the phenomena of CLT, previous work has suggested that a negative influence of ageing on the efficacy of CLT may exist (Hinder, Schmidt, Garry, Carroll, & Summers, 2011), questioning the likelihood of success of such interventions in aged populations.

An alternative clinical approach used more widely to assist in restoring bilateral functional capacity in less severe cases of neurological disorders or limb weakness, in which an ability to perform bilateral movements is to some degree still preserved, is mirror training (MT). Within MT a patient is provided with mirror-visual feedback (MVF) of their intact (uninjured) limb superimposed over their affected limb. During the execution of a bilateral movement task, in which the affected limb is moved as much as possible concurrently with the unaffected limb (i.e., the patient attempts to move both limbs but the affected limb may make lower amplitude or less forceful movements due to its dysfunction or injury), concurrent provision of MVF has been shown to result in greater improvements in the affected limb compared to when training is performed without it (for an overview see Thieme, Mehrholz, Pohl, Behrens, & Dohle, 2012).

Although traditionally used within bilateral training protocols, MT has recently been suggested as an effective tool during unilateral training protocols with the aim of enhancing CLT to the untrained limb. Evidence suggests that, at least in younger adults (Lappchen et al., 2012; Nojima et al., 2012), cross-limb adaptations were facilitated by MT. In light of the aforementioned clinical relevance of CLT, more research is thus warranted to investigate whether combining *unilateral* training programmes with MVF also has the potential to improve *bilateral* performance outcomes in older adults. In addition, future experiments should investigate the neural underpinnings of MT which, due to the diversity of experimental and methodological setups across previous studies, remain incompletely understood. Such knowledge about the neural basis of the phenomenon seems crucial if MT is to be fully exploited as a potential treatment or intervention tool in the ageing population.

1.2. Project aim

The primary aim of the present work was to consolidate previous findings and to provide evidence in order to test current theories regarding both the behavioural and neurophysiological outcomes, together with the neurophysiological underpinnings, of MT.

Specifically, the thesis aimed to study the following:

1. In light of the substantial methodological variations used across previous studies (which may in part account for the differing conclusions across extant studies), the initial aim was to investigate what factors (e.g., nature of movement task, type of visual feedback) have important impacts on the

efficiency of MT. Specifically, this aim attempted to elucidate a better understanding of the way in which MVF may mediate neural changes in the networks innervating the passive limb (i.e., the limb hidden behind the mirror) when the contralateral limb undertakes repeated motor actions. Developing an understanding of the neural basis of the MVF phenomenon and establishing a direct link between neural correlates and motor performance in the context of MT seems crucial to ultimately optimise the use of MVF as a potential treatment or intervention tool (study 1 and follow-up studies 1-3).

2. A secondary goal was to further study the effect of MVF on motor learning and CLT (study 2 and study 3). Again, attempting to consolidate the few previous findings the aim was to investigate the extent to which MVF can reliably contribute to, and further enhance, CLT, and to also identify the underlying neurophysiological changes that correlate with behavioural outcome measures (study 3).
3. Embedded within both of these aims was the third and final aim to study how ageing and age-related changes in brain structure and function alter the effects of MVF on neurophysiological parameters (e.g., corticospinal excitability) (study 1 and study 3) or behavioural outcome measures (i.e., CLT) (study 3).

CHAPTER 2: LITERATURE REVIEW

2.1. Overview

The initial part of this chapter will familiarise the reader with the technique that will be used in the current project to assess dynamic changes within the human motor system. Subsequently, to give a clear understanding of the rationale for the current research project, a review will be provided of the literature existing at the time of commencement of the current project in the areas of MT and CLT in both young and older individuals. Specifically, current models describing the well-known phenomenon of CLT will be described, together with potential sites of adaptation within the human motor system. Information about the behavioural and neurophysiological effects and the potential underlying mechanisms of MT will then be provided. Finally, I will discuss the implications for a potential use of MT to augment CLT in general and in the older population specifically.

2.2. Investigation of the motor cortex using non-invasive techniques

2.2.1. Primary motor cortex

Everyday activities, such as tying shoelaces, writing letters or driving a car, are highly skilled, automated actions, which require considerable learning early in life. One of the main regions of the brain that initially enables us to learn, and subsequently execute voluntary motor tasks, automated or not, in a controlled and accurate way is the primary motor cortex (M1), which is located in the precentral gyrus (Broadmann area 4). The surface area of M1 is organised in a somatotopic way, such that specific regions of the M1 represent, and thus control, particular regions of the body. Known as the human motor homunculus, this somatotopic representation is not static, but rather quite dynamic and changeable. Such changes are made possible by an adaptive process called *neural plasticity*, which contrary to previously held views, is preserved to some extent throughout one's lifetime (Boroojerdi, Ziemann, Chen, Buterfisch, & Cohen, 2001; Dayan & Cohen, 2011; Hallett, 2007; Seidler et al., 2010; Swinnen et al., 2010; Voelcker-Rehage, 2008).

Neural plasticity of the human M1 can be investigated using a variety of both invasive and non-invasive recording and stimulation techniques. Functional neuroimaging techniques, such as functional magnetic resonance imaging (fMRI) or positron emission tomography (PET) are able to provide good spatial information about the brain networks involved in specific functions, and how these networks may evolve in the face of environmental change. However, these techniques lack temporal resolution (Hallett, 2007). Transcranial stimulation techniques, such as transcranial electrical stimulation (TES) or transcranial magnetic stimulation (TMS), on the other hand, provide good spatial and temporal information about the dynamic

changes in the brain networks involved in specific functions, by transiently activating or inhibiting specific brain areas (Hallett, 2007).

2.2.2. Transcranial Magnetic Stimulation

TMS is a safe, non-invasive brain stimulation technique that has been used with increasing regularity in the last two decades to assess neurophysiological functions and probe the dynamics of excitatory and inhibitory circuits in the brain (Chen, 2004; Ferbert et al., 1992; Hallett, 2007; Kujirai et al., 1993; Reis et al., 2008). It was first introduced in 1985 by Anthony Barker, and quickly became a popular alternative to TES (Barker & Jalinous, 1985). As TES involves the induction of a high-voltage electric shock (Barker & Jalinous, 1985) it is a rather uncomfortable and painful technique to stimulate motor areas over the brain. TMS, on the other hand, uses electromagnetic induction as a highly effective painless way to generate suprathreshold current in the brain and study brain excitability in a similar way to TES (Rossini et al., 1994). TMS involves a strong, short-lasting, current passing through the windings of a copper wire coil positioned over the scalp, which produces a brief magnetic field. As intense magnetic fields are not impeded by the skull, the magnetic stimulation induces a perpendicular electrical current in the underlying cortical tissue, which leads to an activation of corticospinal neurons beneath the stimulated area (Ferbert et al., 1992; Kujirai et al., 1993; Rothwell, 1997). There are a number of different designs of stimulation coils, with the shape determining how focussed the induced current will be. The most focal cortical activation is achieved using a figure-of-eight coil, and the response greatest when positioning the coil to evoke anterior-posterior current direction over the skull (Hallett, 2007). When stimulating over M1, the TMS-induced electrical current depolarises those

corticospinal neurons, whose axons are aligned with the current flow. A series of descending volleys will subsequently be evoked, travelling through the corticospinal tract and synapsing onto motor neurons, where the peripheral muscle group related to the part of the stimulated cortex will be activated (Chen, 2000; Rothwell, 1997). The measurable response in the form of an involuntary muscle contraction provides a quantification of corticospinal excitability (CSE) at the time of stimulation (Di Lazzaro et al., 2004; Rothwell, 1997). The muscle response is recorded using surface electromyography (EMG), and referred to as a motor evoked potential (MEP).

MEPs consist of a number of direct and indirect waves elicited in response to the magnetic pulse (Reis et al., 2008). Direct waves (D-waves) represent the initial descending volleys that are created by direct activation of corticospinal neurons, and are usually only apparent at high TMS intensities or with electrical stimulation (Di Lazzaro et al., 2004; Reis et al., 2008; Rothwell, 1997). D-waves are succeeded by a number of indirect waves (I-waves), representing the indirect synaptic activation of corticospinal neurons as a result of depolarising excitatory interneurons (Di Lazzaro et al., 2004; Reis et al., 2008; Rothwell, 1997). TMS tends to predominantly depolarise interneurons leading to several I-waves, with only limited influence from direct corticospinal depolarisation (Reis et al., 2008; Rothwell, 1997); ‘early’ I-waves result from a single interneuron (I1 waves) whereas ‘later’ I-waves, occurring a few milliseconds after the I1 wave (e.g., I2, I3 etc.), represent descending volleys via two or more interneurons.

2.2.2.1. Single-pulse paradigms

Single-pulse stimulation over the M1 produces MEPs in the target muscle group, which represent the net facilitatory effect of the TMS pulse and thus give an insight into CSE. As MEP amplitudes demonstrate a considerable amount of inter- and intra-trial variability, due to participant-related factors such as muscle activation or alertness as well as experimenter-related factors such as coil handling, those factors must be controlled for and sufficient MEP amplitudes must be collected and subsequently averaged (Carroll, Riek, & Carson, 2001; McDonnell & Ridding, 2006). Another important factor to control for in order to obtain reliable results is the consistent placement of the TMS coil at the position over the M1 that most effectively induces MEPs in the contralateral peripheral muscle of interest. This optimal position is termed the motor hotspot. Stimulation of the motor hotspot is used to determine the motor threshold of the target muscle, which represents the interneuronal membrane excitability projecting to the M1, and corticospinal and motor neuron excitability (Kobayashi & Pascual-Leone, 2003). Examining the motor threshold in a quiescent muscle is called resting MT (rMT). It describes the minimum stimulation intensity needed to evoke a peak-to-peak amplitude of 50μV (for rMT) in a target muscle) in 3 out of 5 consecutive trials (Carroll et al., 2008; Hinder et al., 2011; Kujirai et al., 1993; Rossini et al., 1994).

In addition to rMT, other properties of the MEP can be used to elucidate the corticospinal function of the individual tested (Chen, 2000). Amongst those properties is the latency of the MEP, representing the corticospinal conduction time, the MEP amplitude, which is positively related to the stimulus intensity and the excitability of the corticospinal tract, as well as the duration of the corticospinal silent

period following an MEP evoked in an active muscle, depicting cortical and spinal inhibition.

2.2.2.2. Paired-pulse paradigms

While single-pulse stimulation is used to investigate (net) CSE of projections from the stimulated cortical region, paired-pulse paradigms are utilised to investigate intra- and inter-regional neurophysiological mechanisms and how these can change prior to, during or subsequent to the execution of motor tasks. Within such protocols, the delivery of a conditioning stimulus (CS) prior to a test stimulus (TS) to either the same or different cortical regions (using one or two TMS coils, respectively) results in activation of various inhibitory and/or facilitatory cortical mechanisms, which have a subsequent effect on the resultant MEP amplitude elicited from the TS pulse (Ferbert et al., 1992; Kujirai et al., 1993). Whether the CS causes a facilitation or suppression of MEP amplitude depends on the intensity of the stimulus as well as the duration of the interstimulus interval (ISI) between the CS and the TS (Chen, 2004; Ferbert et al., 1992; Kujirai et al., 1993; Reis et al., 2008). As learning occurs, motor training protocols are likely to modify the quantity and interaction of motor cortical inhibitory and facilitatory synapses, with the subsequent measurable change in net corticospinal output representing contingent cortical mechanisms that support training-related behavioural changes.

2.2.2.2.1. Single-coil techniques

Local intracortical inhibition can be studied by delivering a subthreshold CS (approximately 70-80% rMT) prior to a suprathreshold TS (Garry & Thomson, 2009;

Kujirai et al., 1993). At an ISI of 1-6 ms the motor response elicited by the regular TS (approximately 110-120% rMT) is *reduced* by the CS (Garry & Thomson, 2009). This common TMS tool is referred to as short interval intracortical inhibition (SICI) and was first reported by Kujirai et al. (1993). The CS is thought to activate low threshold inhibitory circuits, which utilise the neurotransmitter gamma amino-butyric acid ($GABA_A$), resulting in synaptic inhibition of higher-threshold circuits that are targeted by the test pulse (Garry & Thomson, 2009). While short ISIs (≤ 1 ms) presumably suppress D-waves, longer ISIs (2-6 ms) supposedly suppress I-waves (Fisher, Nakamura, Bestmann, Rothwell, & Bostock, 2002; Hanajima et al., 2003). SICI is commonly reported as a ratio of the MEP amplitude elicited following paired-pulse stimulation compared to the MEP amplitude elicited following single-pulse stimulation, with smaller values indicating stronger inhibition.

Considering SICI to play a functional role in motor control, changes can be expected in the way it modulates the output of M1 (measured via the size of the MEP amplitude) depending on the current behavioural state of the motor system (i.e., active or resting). Indeed, SICI has been shown to be significantly reduced in the presence of low-level voluntary contractions (Ridding, Taylor, & Rothwell, 1995), to be associated with motor learning (Liepert, Classen, Cohen, & Hallett, 1998; Perez, Wise, Willingham, & Cohen, 2007), but also to be altered in different neurological disorders, such as Parkinson's disease (Ridding, Inzelberg, & Rothwell, 1995) and Alzheimer's disease (Liepert, Dettmers, Terborg, & Weiller, 2001). Such findings support the idea of a movement-related modulation of SICI and underline its potential contribution to training-induced behavioural changes.

Delivering the CS 6-30 ms prior to the regular suprathreshold TS activates a population of excitatory interneurons, thus facilitating the elicited MEP amplitude (relative to the size from single pulse stimulation) via an increased overall corticospinal output (Kujirai et al., 1993; Reis et al., 2008; Ziemann, Rothwell, & Ridding, 1996).This mechanism is referred to as intracortical facilitation (ICF) and potentially modulated through the activation of glutamatergic circuits within M1 (Ziemann, 2004). As ICF requires higher CS intensities (80% rMT and more) and is more affected by the current direction produced by the TMS pulse, it is thought to be produced by separate mechanisms and different neuronal populations than SICI (Epstein, Wassermann, & Ziemann, 2008; Strafella & Paus, 2000; Ziemann et al., 1996).

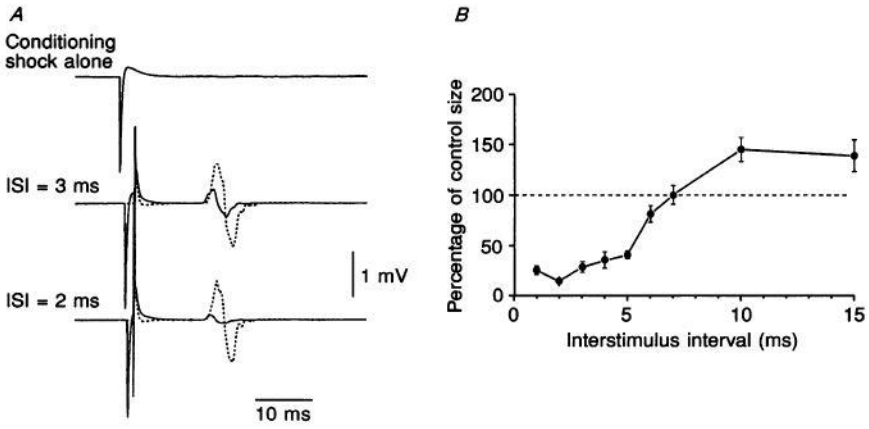

Figure 2.1. Technique of producing short intracortical inhibition and intracortical facilitation.

A) from top to down: conditioning pulse alone, conditioning pulse 3 ms prior to test stimulus, conditioning pulse 2 ms prior to test stimulus. The MEP elicited by the sole test stimulus is indicated with dotted lines. B) illustration of the average effect of paired pulse stimulation on MEPs at different ISIs. (adjusted from Kujirai et al., 1993)

2.2.2.2.2. Double-coil techniques

Delivering two magnetic pulses through two *separate* coils enables the connectivity between different cortical structures to be assessed. Those physiological interactions can have an inhibitory or a facilitatory influence on M1 excitability, and may be assessed for different structures across hemispheres (e.g., left M1 – right M1, left premotor cortex – right M1) or within one hemisphere (e.g., left premotor cortex – left M1) (for an overview see Reis et al., 2008). Similar to the aforementioned paradigms, whether a paired-pulse protocol produces suppression or facilitation of the elicited MEP amplitude depends on several parameters, such as ISI and stimulus intensity.

In 1992, Ferbert et al. were the first to report that the application of a CS over one M1 modulated the MEP amplitude elicited by the following TS on the contralateral M1 (Ferbert et al., 1992). The authors found that the delivery of two suprathreshold TMS pulses (130% rMT) at intervals of 6-50 ms resulted in interhemispheric inhibition (IHI), regardless of whether the target muscle was in a quiescent or activated state. As TES-induced MEPs and spinal excitability as measured by H-reflexes were uninfluenced by the CS (Ferbert et al., 1992; Ni et al., 2009), IHI was concluded to take place at the level of the cerebral cortex (Chen, 2004; Reis et al., 2008). Additional findings indicated reduced or absent IHI in patients with abnormalities to the corpus callosum (Meyer, Roricht, Voeinsiedel, Kruggel, & Weindl, 1995); accordingly this interhemispheric inhibitory effect was deemed to be mediated through transcallosal pathways. More specifically, IHI involves the activation of transcallosal excitatory (i.e., glutamatergic) pathways that synapse onto local inhibitory circuits within the target hemisphere leading to a decrease in cortical

net output (Ferbert et al., 1992; Ni et al., 2009; Reis et al., 2008). Similar to SICI, IHI is reported as a ratio of the MEP amplitude elicited following paired-pulse stimulation compared to the MEP following single-pulse stimulation, with smaller values indicating stronger present IHI. While IHI can be observed over a range of ISIs from 6-50 ms, it is most clearly elicited at two different ISIs, which are supposed to be mediated by different mechanisms (Ferbert et al., 1992; Reis et al., 2008). IHI measured at 10 ms ISI is thought to represent the activation of direct transcallosal pathways between the two motor cortices, whereas IHI measured at an ISI of 40 ms is purported to reflect activation of a more widespread inhibitory system involving the activation of various motor-related cortical areas, such as the premotor or somatosensory cortex (Chen, Yung, & Li, 2003; Ni et al., 2009).

As transcallosal projections appear to contribute significantly to modulated interactions between the two motor cortices at rest and during motor tasks (Ferbert et al., 1992; Meyer, Roricht, Voneinsiedel, Kruggel, & Weindl, 1995), IHI has been proposed to play an important role in the execution of uni- and bilateral motor actions (for an overview see Carson, 2005; Perez, 2012). A modulation of IHI, with increases onto the non-responding hand and decreases onto the responding hand, is thereby thought to underlie fast responses while minimising movements with the wrong hand or mirror activity. Support for such activity-dependent changes of IHI and its functional role in motor control processes comes from previous studies that have found activity-dependent changes prior and during the performance of motor tasks (Duque et al., 2007; Muellbacher, Facchini, Boroojerdi, & Hallett, 2000; Murase, Duque, Mazzocchio, & Cohen, 2004; Perez & Cohen, 2008) as well as following a motor learning paradigm (Hortobagyi et al., 2011; Perez, Wise, et al., 2007). Moreover, the degree of IHI has been shown to be reduced in musicians (Ridding,

Brouwer, & Nordstrom, 2000), and partially affected by ageing (Talelli, Waddingham, Ewas, Rothwell, & Ward, 2008).

2.3. Cross-limb transfer

2.3.1. Definition and evidence

It has long been understood that repetitive unilateral motor activity influences the performance of the active (trained) limb as well as the homologous action in the opposite limb. This phenomenon was first reported in the scientific literature in 1894 (Scripture et al, 1894), and is nowadays commonly referred to as either “cross-education” (Hortobagyi, 2005; Zhou, 2000) or “cross-limb transfer” (Hinder et al., 2011; Lee, Hinder, Gandevia, & Carroll, 2010). Although most of the previous research has since been conducted focussing on strength protocols (Farthing, Chilibeck, & Binsted, 2005; Hortobagyi et al., 2011; Kidgell, Stokes, & Pearce, 2011; Munn, Herbert, Hancock, & Gandevia, 2005), other studies have also demonstrated the existence of cross-limb transfer (CLT) in a variety of simple and complex training paradigms, such as ballistic motor tasks (Carroll et al., 2008; Hinder, Carroll, & Summers, 2013; Lee et al., 2010), or sequential motor learning tasks (Perez & Cohen, 2008; Perez, Tanaka, et al., 2007). Although shown to occur irrespective of limb dominance or gender (Hortobagyi, 2005; Zhou, 2000), CLT appears to be confined to the homologous muscle group in the contralateral limb (Hortobagyi, Lambert, & Hill, 1997; Hortobagyi, Scott, Lambert, Hamilton, & Tracy, 1999) and appears most pronounced when the type of movement of the untrained limb matches the one that was practiced with the opposite limb (Hortobagyi et al., 1997; Zhou, 2000). According to previous reviews the average performance gain obtained in the untrained hand varies between approximately 7% (Carroll et al., 2006) to 20% (Hortobagyi, 2005), however, the magnitude of CLT has been found to be enhanced when practicing eccentric muscle contractions compared to concentric or isometric

contractions (Hortobagyi et al., 1997; Hortobagyi et al., 1999; Zhou, 2000) and in the presence of additional electrical stimulation applied to the active limb during unilateral training (Hortobagyi et al., 1999). Interestingly, performance improvements in the untrained limb occur in the absence of hypertrophy in the corresponding muscle (Farthing, Borowsky, Chilibeck, Binsted, & Sarty, 2007; Farthing et al., 2005) and even under complete absence of voluntary activation in the same limb (measured via EMG) (Hortobagyi, Taylor, Petersen, Russell, & Gandevia, 2003). Considering those facts and the presence of training-induced CSE increases in the hemisphere *ipsilateral* to the active limb (Carroll et al., 2008; Hinder et al., 2011; Lee et al., 2010), CLT is suggested to be predominantly of cortical, rather than subcortical, spinal or muscle origin (Carson et al., 2004; Hortobagyi et al., 2011; Hortobagyi et al., 2003; Zhou, 2000). Considering unilateral training interventions to be able to attenuate strength loss in an immobilised limb with (Magnus et al., 2013) or without a fracture (Pearce, Hendy, Bowen, & Kidgell, 2013), CLT appears, despite its relatively small effect, to be a clinically and functionally meaningful tool for the treatment of unilateral or orthopaedic disorders within a rehabilitation setting.

2.3.2. Potential sites of adaptation

Specific potential locations for CLT-related adaptations following strength training protocols have previously been considered by several reviews, pointing to muscle-, spinal-, and/or cortical-level adaptations (Carroll et al., 2006; Hortobagyi, 2005; Zhou, 2000). Moreover, potential mechanisms by which CLT may occur have been discussed, generally referring to adaptations in the system controlling the active limb and/or the inactive limb. The following section will discuss those potential sites and

the underlying mechanisms involved in the phenomenon of CLT in more detail, primarily focussing on strength training protocols.

2.3.2.1. Muscle level adaptations

Previous CLT-related research (Hortobagyi et al., 1996; Moritani & Devries, 1979; Ploutz, Tesch, Biro, & Dudley, 1994) has failed to demonstrate substantial muscle adaptations in the limb contralateral to the trained limb, such as hypertrophy or changes in muscle enzyme concentration, which are considered an important contributor to strength gain in a trained muscle (Baldwin & Haddad, 2001). Although EMG activity can be observed in both the homologous and non-homologous muscles contralateral to the active limb, intensities of approximately 10-15% of that observed in the active limb (Hortobagyi, 2005) are usually considered to be too small to drive muscle-level adaptations in the untrained limb. Moreover, increases in strength are specific to the homologous muscle (Hortobagyi et al., 1997; Hortobagyi et al., 1999), and even occur in the absence of EMG activity (Hortobagyi et al., 1997; Hortobagyi et al., 2003). Summarising the evidence, it appears that muscle-based mechanisms (i.e., adaptations at the muscle level) are an improbable primary site of adaptation for CLT-related behavioural changes (Carroll et al., 2006; Hortobagyi, 2005; Zhou, 2000). However, it is important to note that some adaptation at the muscle level cannot be completely ruled out, as current techniques may simply not be sensitive enough to detect muscular changes (Carroll et al., 2006).

2.3.2.2. Spinal level adaptations

Different networks within the spinal cord have been suggested to influence motor output and be modified through strength training (Carroll et al., 2006; Carroll, Selvanayagam, Riek, & Semmler, 2011). Support for involvement of the spinal cord in CLT comes from studies using electrical stimulation, resulting in artificial activation of muscles. Such electrical stimulation has previously been shown to lead to CLT effects in the unstimulated limb, despite a lack of involvement of cortical control (Hortobagyi, 2005; Hortobagyi et al., 1999; Zhou, 2000). Those findings are contradictory to studies that have previously reported strength-related CLT-effects in the absence of a significant change in H-reflexes in the untrained limb (Dragert & Zehr, 2011; Lagerquist, Zehr, & Docherty, 2006). However, it is worth noting that the measurement of H-reflexes will not capture all afferent pathways (Ia interneurons), and that some activation of the spinal through group III/IV afferents cannot be ruled out entirely. As different spinal mechanisms to those assessed by way of H-reflex responses might thus potentially contribute to CLT-effects, more research comparing changes in spinal cord circuits of the trained and the untrained limb is needed to make a more precise statement with regard to potential CLT-related adaptations at the spinal level (Carroll et al., 2006).

2.3.2.3. Cortical level adaptations

Although adaptations at both the spinal and muscle level cannot be completely ruled out, a major contribution to CLT-effects seems to stem from adaptations at the cortical level. Two theoretical models of cortical plasticity describe the specific cortical sites that might contribute to CLT. The first model posits that adaptations in

the control system of the active limb, accessible by the opposite (i.e., inactive) limb, are primarily responsible for CLT. Referred to as the “callosal access model”, the model was first introduced by Taylor and Heilman (1980). According to this model, practice-induced motor engrams are stored at a site within the central nervous system, which is either accessible by the untrained limb (i.e., storage within the trained hemisphere), or by both the trained and the untrained limb (i.e., storage at a central site).

The second model is referred to as the “cross-activation model” and was first introduced by Parlow and Kinsbourne (1989). According to this model, unilateral training leads to bilateral activation (i.e., cross-activation) and adaptations in both systems (i.e., the hemisphere controlling the active limb as well as the hemisphere controlling the inactive limb), subsequently leading to CLT. These models are not necessarily mutually exclusive (Carroll et al., 2006; Lee & Carroll, 2007; Parlow & Kinsbourne, 1989), but may depend on the type of the unilateral movement task (Lee et al., 2010). Whereas training protocols requiring strength efforts may induce changes in accordance with the cross-activation model, training paradigms involving complex sequencing and/or sensorimotor integration may most likely evoke adaptations in alignment with the callosal access model.

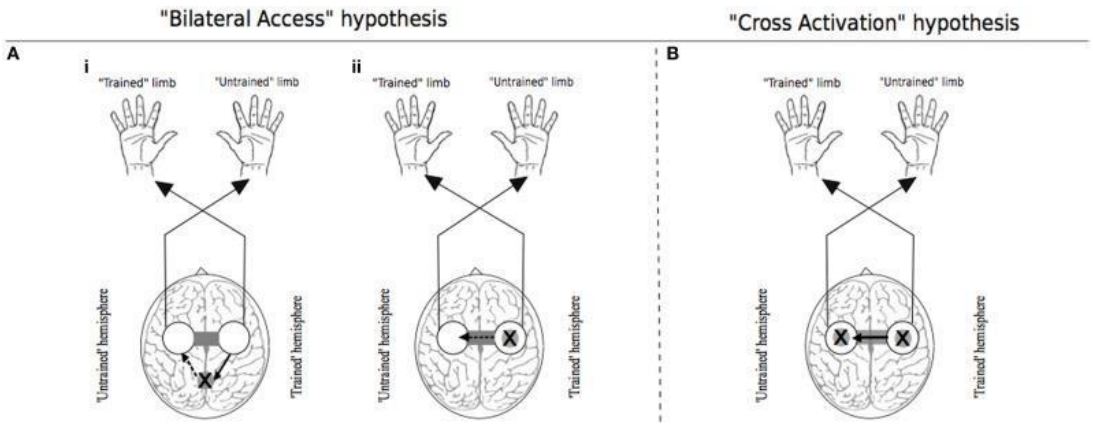

Figure 2.2. Traditional theoretical models of cross-limb transfer

The “X” represents the putative locus of training-related adaptations. A) Adaptations according to the “Bilateral Access” Theory occur: (i) cortical, or subcortical, areas that project bilaterally, (ii) in motor areas of the trained hemisphere. B) Adaptations according to the “Cross Activation” Theory occur: in motor areas of the untrained hemisphere. (adjusted from Ruddy & Carson, 2013)

2.3.3. Cross-activation as the mechanism underlying cross-limb transfer

As the current research does not focus on complex sequencing and/or sensorimotor integration training paradigms, but rather on simple motor learning tasks including ballistic motor learning (which shares some of the neural substrates of strength training, (Selvanayagam, Riek, & Carroll, 2011), the focus will predominantly be put on further illuminating the neurophysiological mechanisms that potentially underpin the cross-activation model.

2.3.3.1. Evidence for ipsilateral motor cortex activation

Cross-activation (i.e., activation of the hemisphere ipsilateral to an active limb) as a result of forceful unilateral contractions has been demonstrated in imaging studies

showing increased blood flow (Cramer, Finklestein, Schaechter, Bush, & Rosen, 1999; Dettmers et al., 1995; Kobayashi & Pascual-Leone, 2003) and TMS studies showing increased corticospinal activity (Hortobagyi et al., 2003; Muellbacher et al., 2000; Perez & Cohen, 2008; Stinear, Walker, & Byblow, 2001) during task activation. The spill-over of unintended activity from the active to the inactive M1 (i.e., motor irradiation), which in the past has been shown to occasionally evoke mirror movements and augmented background EMG in the limb contralateral to the active limb (Cernacek, 1961; Todor & Lazarus, 1986), has been hypothesised as one potential mechanism underpinning the behavioural observations of CLT. However, as cross-activation has also been observed without present mirror movements or EMG increases in the hemisphere ipsilateral to the active limb (Carson, Welsh, & Pamblanco-Valero, 2005; Hortobagyi et al., 2011), it is more likely that actual crossed cortical activation of the M1, rather than incidental “motor-irradiation”, is responsible for cross-activation. Findings in support of this assumption have demonstrated an ipsilateral CSE increase with increasing contraction intensities (Hortobagyi et al., 2003; Muellbacher et al., 2000; Stinear et al., 2001), as well as a relationship between the degree of CLT and performance gains in the trained limb (Carroll et al., 2006; Zhou, 2000). It should be noted, however, that such plastic changes in the ipsilateral hemisphere are unlikely to be restricted to M1. Rather, as suggested by imaging studies, areas upstream of M1, such as the supplementary motor area, cingulate motor area and prefrontal cortex (Dettmers et al., 1995; Farthing et al., 2007; Kawashima et al., 1993) might also contribute to and modify cross-activation, respectively. Finally, additional involvement of subcortical areas, such as the basal ganglia or cerebellum, in promoting cross-activation, cannot currently be entirely ruled out (Carroll et al., 2006).

2.3.3.2. Mechanisms underlying ipsilateral motor cortex activation

Considering cross-activation to occur at a cortical, rather than subcortical or spinal level, there are different sites that contributing pathways could originate from. Despite a number of descending pathways leaving the cortex either activating muscles on the same (i.e., ipsilateral) side, or innervating homologous muscles bilaterally, such pathways are unlikely to be the main contributor to CLT (Carroll et al., 2006; Hortobagyi, 2005). Rather, it seems plausible that excitatory and inhibitory *interhemispheric pathways* between the cortices may primarily be involved in the process of cross-activation and subsequent CLT effects (Carroll et al., 2006; Hortobagyi, 2005). IHI via the corpus callosum, the anatomical structure that connects both M1s and most likely mediates cross-activation, has previously been demonstrated to be influenced by voluntary muscle contractions (Howatson et al., 2011; Muellbacher et al., 2000; Perez & Cohen, 2008). In addition to a change in IHI, voluntary contractions have also been shown to concurrently modulate ipsilateral excitability, leading to increases in activity of corticospinal output paths (i.e., MEPs) and a reduction in SICI (Perez & Cohen, 2008).

Such increased ipsilateral M1 excitability has previously been shown to occur as a consequence of *short-term* unilateral ballistic motor training paradigms in which CLT of behaviour is observed (Carroll et al., 2008; Hinder et al., 2013; Lee et al., 2010). Perez et al. found a decrease of both IHI (from the active to the inactive hemisphere) and SICI (in the ipsilateral hemisphere) as a consequence of a unilateral sequential motor learning task. Interestingly, only the degree of IHI modulation was found to be related to CLT-effects (Perez, Wise, et al., 2007).

On a similar note, studying the effects of a *long-term* strength training protocol, Hortobagyi et al. (2011) recently showed a correlation between strength increases in the untrained limb (i.e., CLT) and reduced IHI from the active M1 to the inactive M1, but no relationship between changes in SICI or ICF in the inactive hemisphere and CLT. Apart from ipsilateral CSE increases (Goodwill, Pearce, & Kidgell, 2012; Hortobagyi et al., 2011; Kidgell et al., 2011) and decreases in SICI in the ipsilateral hemisphere (Goodwill et al., 2012), longer-lasting strength training protocols have also been shown to lead to a reduced silent period duration in the same hemisphere (Latella, Kidgell, & Pearce, 2012).

In summary, although it seems likely that homotopic interhemispheric connections and their effect on interneurons involved in intracortical excitability might mediate the behavioural changes associated with CLT (Hortobagyi, 2005), based on research that demonstrated CLT in patients with complete agenesis of the corpus callosum (Meyer et al., 1995), it cannot be assumed that such a direct connection between both M1s via the corpus callosum is the *exclusive* source for cross-activation. Rather, it is possible that the increase in ipsilateral M1 excitability might also arise independently from activation in the opposite M1, or from motor planning areas upstream of M1 (e.g., via different centres within the motor network that connect bilaterally onto M1s) (Carroll et al., 2006).

2.3.4. Cross-limb transfer in the context of ageing

An approach to treat neurological or orthopaedic disorders and facilitate bilateral behavioural benefits in response to unilateral training paradigms is particularly appealing for the older population, in which age-related mobility deficits due to stroke

or fall-related incidents may preclude the sufficient use of bilateral motor training programmes. However, regarding the ability of older adults to exhibit CLT, previous studies have shown inconsistent results. While some studies demonstrated preserved CLT in older adults (Langan & Seidler, 2011; Panzer, Gruetzmacher, Fries, Krueger, & Shea, 2011; Seidler, 2007), others reported a reduction in the extent to which CLT was manifested relative to that observed in younger adults (Bemben & Murphy, 2001; Hinder et al., 2011). A potential reason for the lack of consistency in results could be due to differences in task complexity. While CLT appears to be absent in older adults in simple ballistic tasks (Hinder et al., 2011), the preserved effects in more complex, usually visuomotor tasks (Panzer et al., 2011; Seidler, 2007) might reflect transfer of a cognitive, rather than of a motor, component of the adaptation. Moreover, asymmetries (that are not observed in younger adults) may exist in the transfer of performance in older adults depending on the direction (i.e., from the dominant to non-dominant hand or vice versa); indeed, while Hinder et al. (2011) clearly showed an absence of CLT in older adults following dominant limb training, a follow up study (Hinder et al., 2013) showed that older adults were able to exhibit CLT following non-dominant limb training. Considering decreased proprioceptive abilities in older adults (Goble, Coxon, Wenderoth, Van Impe, & Swinnen, 2009), the presence or absence of CLT might also be affected by whether learning a task is influenced (e.g., ballistic motor tasks) or uninfluenced (e.g., visuomotor tasks) by proprioception (Pipereit, Bock, & Vercher, 2006). Finally, it is possible that older adults' tendency to exhibit bilateral M1 activation to facilitate / support the learning of unilateral tasks (Bodwell, Mahurin, Waddle, Price, & Cramer, 2003; Mattay et al., 2002; Ward & Frackowiak, 2003) might subsequently interfere with the process of CLT, depending on the whether the transfer of the acquired

motor skill potentially relies on (disposable) M1 activation (i.e., simple motor skill) or not (i.e., complex motor skills) (for an overview see Tanji, 2001).

2.3.5. Summary

Bilateral performance gains as a consequence of unilateral training are a well-documented phenomenon with great clinical potential. Although the specific potential locations for CLT-related adaptations are still not completely understood, it seems likely that processes contributing to CLT predominantly take place on a cortical, rather than a spinal or a muscle level. With regard to the specific cortical site, current models either refer to adaptations in the control system of the active limb or the inactive limb as a requirement for CLT. Most likely the exact location appears to be dependent on the type of unilateral movement task (Lee et al., 2010), with the learning of strength-accentuated tasks resulting in changes in the untrained hemisphere and that of sequential motor tasks modifying the active hemisphere. Moreover, while CLT has been demonstrated across an array of different movement tasks in younger adults, older adults' capacities to benefit from unilateral training protocols appear to be associated with the complexity of the performed motor task, with the exact mechanisms underlying age-related diverse effects being poorly understood. However, as unilateral training protocols are particularly appealing in the older population, more research is warranted to further examine age-related differences in CLT, and also to investigate possible ways to enhance and improve this attenuation of CLT.

2.4. Mirror therapy

2.4.1. Definition

Mirror therapy (MT) was first introduced by Ramachandran and Rogers-Ramachandran in the mid-1990s (Ramachandran & RogersRamachandran, 1996) as a visual illusion to alleviate phantom limb pain. During MT, a mirror is placed in the patient's midsagittal plane, thus superimposing a mirror image of one limb over the other (obscured), contralateral limb. Moving the unobscured limb gives the impression that the obscured limb is performing the same movement despite it actually remaining quiescent, or indeed absent in the case of an amputee. The use of mirror-visual feedback (MVF) as a successful neurorehabilitation tool has since not only been further established for the treatment of phantom limb pain (Chan et al., 2007; Darnall, 2009), but has also been adopted for the treatment of unilateral pain or movement disorders, such as hemiparesis after stroke (Altschuler et al., 1999; Thieme et al., 2012; Yavuzer et al., 2008), chronic regional pain syndrome (CRPS) treatment (McCabe et al., 2003; Ramachandran & Altschuler, 2009), or reduced mobility after wrist fracture (Altschuler & Hu, 2008).

2.4.2. Mirror-visual-feedback-induced behavioural effects

By virtue of the fact that MT was developed primarily as a neurorehabilitation tool most of the previous MT research has involved case studies or relatively small groups of clinical populations with specific neurological disorders. Focussing on behavioural change as the primary outcome measure, these studies have generally utilised subjective measures, such as pain reduction, or subjective measures of motor improvements, such as functional independence (for an overview see

Ramachandran & Altschuler, 2009; or Thieme et al., 2012) to quantify the efficacy of the technique. The aforementioned studies focussing on behavioural effects appear to show reasonably beneficial and consistent effects of MT, such as reduction of pain in phantom limbs (Chan et al., 2007; Darnall, 2009; Ramachandran, Rogersramachandran, & Cobb, 1995) and acute Complex Regional Pain Syndrome (Karmarkar & Lieberman, 2006; McCabe et al., 2003), as well as faster improvement from hemiparesis following stroke (Altschuler et al., 1999; Yavuzer et al., 2008). Indeed, Thieme et al. (2012) in a recent review paper examined previous behavioural studies, particularly focussing on the effectiveness of MT with regard to improved motor functions, daily activities, pain and visuospatial neglect in patients after stroke. According to this review, MT appears to have a significant effect on motor function, as well as positive effects on activities of daily living and non-motor symptoms such as pain; effects that were retained up to 6 months after cessation of the intervention. Despite the call for further research comprising larger sample sizes, the review also supports the potential effectiveness of MT within stroke rehabilitation, at least when used as an *adjunct to commonly employed rehabilitation methods* (Thieme et al., 2012). This particular conclusion is consistent with another previous article, reviewing the effects of MT when used as a tool within pain treatment (Moseley, Gallace, & Spencec, 2008). Specifically, Moseley et al. (2008) conclude that MT, when applied on its own, failed to evoke any greater immediate pain relief than motor imagery. The authors, however, suggest that MT may provide an additional benefit when used as a daily intervention embedded within a motor imagery programme (Moseley et al., 2008). In summary, MT and MVF seem to have the potential to induce behavioural changes in an array of neurological disorders that can outweigh and outlast those brought about by movement rehabilitation

programmes undertaken with no specific manipulation of visual feedback of the undertaken task.

2.4.3. Mirror-visual-feedback-induced neurophysiological effects

In contrast to the above mentioned studies focussing on subjective behavioural outcomes of MT, considerably less research has focussed on investigating the neural mechanisms underlying the manifested behavioural changes, as well as quantifying behavioural effects using objective measures. Additionally, and contrary to those small-sample-sized but reasonably consistent MVF-induced behavioural effects (Altschuler et al., 1999; Chan et al., 2007; McCabe et al., 2003; Ramachandran et al., 1995), those studies investigating the neural underpinnings of MT have produced equivocal results. One potential reason may be the wide array of imaging and brain stimulation techniques employed by the few studies that have specifically explored the mechanisms underlying MT, including fMRI (Shinoura et al., 2008), TMS (Carson & Ruddy, 2012; Fukumura, Sugawara, Tanabe, Ushiba, & Tomita, 2007; Funase, Tabira, Higashi, Liang, & Kasai, 2007; Garry, Loftus, & Summers, 2005; Lappchen et al., 2012; Nojima et al., 2012), magnetoencephalography (Tominaga et al., 2011), PET (Fink et al., 1999), and electroencephalography (Touzalin-Chretien & Dufour, 2008).

2.4.3.1. Immediate neurophysiological effects

The majority of previous research has investigated the *immediate* neurophysiological effects induced by MT, such as changes in brain activity or excitation. Extant imaging studies have demonstrated a MVF-induced activity increase in areas primarily

ipsilateral to the moving hand, such as the primary visual, somatosensory, and parietal cortex (Dohle, Kleiser, Seitz, & Freund, 2004; Matthys et al., 2009; Wasaka & Kakigi, 2012), dorsolateral prefrontal cortex (Fink et al., 1999), or superior temporal gyrus (Matthys et al., 2009). Assuming such changes in the hemisphere ipsilateral to the moving limb may subserve performance gains in the untrained limb, by way of CLT (Carroll et al., 2008; Garry et al., 2005; Hinder, Schmidt, Garry, & Summers, 2010a, 2010b; Lee et al., 2010), previous studies using non-invasive brain stimulation (NIBS) techniques predominantly focussed on how MVF might mediate activity within the M1 innervating the corresponding passive limb (i.e., the limb that is hidden behind the mirror) (Carson & Ruddy, 2012; Fukumura et al., 2007; Funase et al., 2007; Garry et al., 2005; Kang et al., 2012).

In 2005 Garry et al. were the first to publish a study regarding the underlying neural mechanisms of MT (Garry et al., 2005). Participants were asked to perform a continuous unimanual finger abduction-adduction movement and were provided with different types of visual feedback during the period in which they undertook the motor task. Participants were asked to either fixate on their active or inactive hand, on a centrally aligned mark, or on a mirror reflection of their active hand superimposed over their inactive hand, which was positioned behind the mirror. TMS was applied during task performance to measure changes in CSE in the hemisphere ipsilateral to the moving hand. Consistent with previous research demonstrating bilateral excitability changes due to unilateral movements (Liepert et al., 2001; Muellbacher et al., 2000), results showed a generalised excitability increase in the ipsilateral hemisphere during task performance relative to a resting condition (where both limbs were quiescent). More interestingly, the condition in which participants viewed a mirror image of their moving hand resulted in greater CSE increases in the

M1 ipsilateral to the moving hand, relative to other different visual feedback conditions (e.g. viewing the passive or active hand and fixating a centrally aligned mark). Several subsequent studies have supported those original findings of an enhanced MVF-induced activation in the ipsilateral M1 (Kang et al., 2012; Shinoura et al., 2008; Touzalin-Chretien & Dufour, 2008); however other studies have either only found partial support (Fukumura et al., 2007), or in fact reported quite differing results (Carson & Ruddy, 2012). Although all of these studies suggest that modifying visual feedback during a motor task has the potential to influence the magnitude of CSE changes in the hemisphere ipsilateral to a unimanual movement, there is disagreement as to what extent MT actually confers a *greater* increase in ipsilateral CSE than other feedback conditions.

2.4.3.2. Practice-induced effects

Considerably less research has focussed on investigating the *aftereffects* of MVF with regard to changes in motor performance and brain activity/excitability, when used in combination with training interventions in healthy and neurologically impaired participants (Bae, Jeong, & Kim, 2012; Bhasin, Srivastava, Kumaran, Bhatia, & Mohanty, 2012; Hamzei et al., 2012; Lappchen et al., 2012; Michielsen et al., 2011; Nojima et al., 2012). Regardless of the type of motor task and the duration of the training period respectively, all of these studies revealed a greater improvement in the untrained or affected limb as a result of motor training with MVF compared to motor training without MVF. In contrast to Hamzei et al. (2012), who proposed that the improvements in the untrained limb were related to activity changes in the *active* hemisphere, the majority of studies suggest enhanced MVF-induced excitatory function in the *inactive* hemisphere (expressed as a decrease in intracortical

inhibition) to be related to such performance gains (e.g., Lappchen et al., 2012; Nojima et al., 2012). Furthermore, previous findings demonstrate a training-induced shift in the balance of excitability/activation between both M1s towards the affected hemisphere in neurologically impaired participants, thus suggesting an increased activation in the affected and/or decreased activation in the unaffected hemisphere subsequent to MVF-related training (Bae et al., 2012; Bhasin et al., 2012; Michielsen et al., 2011). In conclusion, such a positive association between behavioural and neurophysiological changes induced by MVF-combined training interventions emphasises the importance of visual feedback modifications for performance improvements in the untrained hand. They further suggest that M1 plasticity, expressed as altered excitatory functions of the M1, seems to be an essential component of MVF-based interventions in both the passive and the active hemisphere (Bae et al., 2012; Bhasin et al., 2012; Hamzei et al., 2012; Lappchen et al., 2012; Michielsen et al., 2011; Nojima et al., 2012).

2.4.4. Mechanisms underlying mirror-visual-feedback-induced changes

In order for MT to be applied most efficiently, it is important to gain a better understanding of the processes that underlie the beneficial behavioural effects of MVF. In this regard, different hypotheses have been put forward to explain the results obtained in healthy and various clinical populations over the last decade (e.g., Garry et al., 2005; Michielsen et al., 2011; Moseley et al., 2008; Nojima et al., 2012; Ramachandran & Altschuler, 2009; Thieme et al., 2012).

One common explanation is based on the existence of mirror neurons (Michielsen et al., 2011; Moseley et al., 2008; Nojima et al., 2012; Ramachandran & Altschuler,

2009), a subset of motor command neurons that discharge not only during action execution, but also during action perception and observation. The mirror neuron system was first proposed following experiments in monkeys conducted by Rizzolatti et al. in the early 1990s (Dipellegrino, Fadiga, Fogassi, Gallese, & Rizzolatti, 1992) and a similar system (i.e., cortical motor areas that are activated during action observation and action execution) has since been suggested to also exist in humans (Rizzolatti & Craighero, 2004). Activation of such an action observation/action execution matching system (Touzalin-Chretien & Dufour, 2008) has previously been shown to lead to increases in CSE (Fadiga, Fogassi, Pavesi, & Rizzolatti, 1995; Stefan et al., 2005), and also appears crucial for action recognition and motor learning or rehabilitation (Buccino, Solodkin, & Small, 2006). MVF might thus be an effective trigger to facilitate action observation and therefore the mirror neuron system, leading to increased cortical and spinal excitability in the inactive hemisphere ultimately leading to behavioural performance gains (Funase et al., 2007; Lappchen et al., 2012; Moseley et al., 2008; Nojima et al., 2012; Touzalin-Chretien & Dufour, 2008). In a similar vein, MT has been described as a variant of motor imagery training (Thieme et al., 2012). Motor imagery, a mental process in which movements are internally simulated without executing them overtly, has been shown to activate neural circuits involved in motor control (Grezes & Decety, 2001), and the occurrence of implicit motor imagery during MT has thus been suggested as one reason for the induced neurophysiological and behavioural changes (Michielsen et al., 2011; Nojima et al., 2012).

An alternative hypothesis suggests MVF might be helpful in restoring the congruency between vision (afferent information) and motor output (efferent information), which is known to be affected in neurological disorders, such as stroke or phantom limb

pain (Ramachandran & Altschuler, 2009). This postulation is based upon the well-known dominance of visual perception over proprioceptive feedback (Brass, Bekkering, & Prinz, 2001; Edwards, Humphreys, & Castiello, 2003; Touzalin-Chretien, Ehrler, & Dufour, 2010), and suggests that increased attention toward the impaired side as well as the provision of an illusory image of an intact limb might lead to reactivation of motor networks within the impaired hemisphere. This explanation has been used to interpret improvements in subjective measurements, such as pain or paralysis, resulting from MVF (Moseley et al., 2008; Tominaga et al., 2011). Finally, MVF has been suggested to facilitate motor recovery in stroke patients by recruiting and partially reviving “dormant” ipsilateral motor pathways that originate in the active hemisphere and innervate the ipsilateral paretic body-side (Ezendam, Bongers, & Jannink, 2009; Ramachandran & Altschuler, 2009).

In summary, the precise underlying neurophysiological mechanisms of the effect of MT are still poorly understood, as is the exact relationship between MVF-induced behavioural changes and neurophysiological effects. Future research thus needs to explore this matter further, while considering that the previously mentioned explanations for the underlying neural effects of MT are most likely not mutually exclusive.

2.4.5. Mirror training in the context of ageing

Although MT has previously been applied and studied in an array of neurological disorders (for an overview see Ramachandran & Altschuler, 2009) which may primarily affect aged populations, to date (i.e., at commencement of this research project) no research has investigated the specific behavioural and

neurophysiological effects of MVF when applied in a healthy ageing population. This is somewhat surprising, given that MT has potential to be applied in non-clinical aged population to aid in motor performance. However, it is important to investigate whether MT-related performance gains remain present when significant structural and functional changes in the brain associated with the process of ageing may affect the underlying process mediating MT. Such age-related brain changes have been shown to influence behavioural aspects of motor performance evident, for example, in older adults' tendencies of increased bilateral activation during the execution of intended unilateral motor tasks (i.e., mirror activity) when compared to younger adults (Hinder et al., 2011; Ward & Frackowiak, 2003). Age-related changes have also been demonstrated to affect the neural control mechanisms underpinning motor performance (Fujiyama, Garry, Levin, Swinnen, & Summers, 2009; Hinder, Fujiyama, & Summers, 2012; Hinder et al., 2011), such as an altered ability to modulate inhibitory mechanisms (Talelli et al., 2008), which is most likely due to a reduced integrity of the corpus callosum as a result of the ageing process (Hoy, Fitzgerald, Bradshaw, Armatas, & Georgiou-Karistianis, 2004).

In addition to the above-mentioned changes, older adults are also known for a greater reliance on visual control and feedback to permit accurate motor performance (Swinnen et al., 1998; Voelcker-Rehage, 2008) as well as an increased use of visual strategies during motor acquisition and performance (Swinnen et al., 2010). An important question is therefore whether older people respond more strongly to MT and benefit more from MVF while performing and learning a motor task than younger people. As stroke and fall-related injuries represent two major concerns when dealing with health issues in older people, such findings would have important implications for the use of specifically tailored MVF-combined interventions

in an ageing population in order to regain a lost independence due to unilateral impaired motor functions.

2.4.6. Summary

After more than a decade of using MT within clinical and research settings, there is still a substantial degree of uncertainty with regard to the exact behavioural and neurophysiological effects of MVF. Even though MT has been shown to provide certain beneficial behavioural effects, the precise underlying neurophysiological mechanisms induced by its use remain rather speculative. One of the reasons for the lack of consensus in findings might be due to differences in methodology used across studies. Differences in task complexity, for example, might have been one influencing factor, ranging from simple (Carson & Ruddy, 2012; Funase et al., 2007; Garry et al., 2005) to more complex motor tasks (Lappchen et al., 2012; Nojima et al., 2012). A lack of standard control conditions further complicates comparisons across different experiments. Specifically, some studies compared the effects of MVF with those obtained from watching either the active or the passive limb (Garry et al., 2005; Tominaga et al., 2011; Touzalin-Chretien & Dufour, 2008), whereas other studies used a comparison of full vision of the two hands (Fukumura et al., 2007; Shinoura et al., 2008). Furthermore, during the MVF-condition, some studies allowed vision of both the active hand and the mirror image of the active hand (Carson & Ruddy, 2012; Garry et al., 2005), whereas other studies prevented bilateral vision by occluding the direct view of the active hand for example via a box (Tominaga et al., 2011; Touzalin-Chretien & Dufour, 2008). Finally, considering the small sample sizes, commonly used in studies within clinical and research settings, future research should aim for more standardised controlled studies with larger

sample sizes. In order to improve the use and increase the success of MT, experiments should predominantly aim at identifying those factors that may have important impacts on the effects of MT, while still trying to provide a direct link between the neural correlates and motor performance. Thus, rather than being purely focused on resolving uncertainties within the theoretical background of the MVF induced changes (i.e., refining theory by targeting existing MVF-related hypotheses), experiments should aim at being clinically significant (i.e., practical use within a rehabilitation and clinical setting) and specifically tailored to the needs of different clinical populations.

2.5. Cross-limb transfer modulation via mirror-visual feedback

The extent to which unilateral training protocols elicit bilateral performance gains is dependent on a variety of factors, such as the nature of the task and the learning environment (Imamizu & Shimojo, 1995; Teixeira, 2000; Zhou, 2000). According to the findings of previous research, CLT also appears to be somewhat negatively affected by ageing (Bemben & Murphy, 2001; Hinder et al., 2011). Considering age-related mobility deficits that may preclude the sufficient use of bilateral motor training programmes, establishing strategies to overcome and counteract previously reported attenuations in CLT seems an appealing and plausible, even necessary, goal for future research in the field of CLT. In this regard, two studies previously examined how modulation of visual feedback during the performance of a variety of complex unilateral motor tasks affected subsequent CLT in younger adults (Lappchen et al., 2012; Nojima et al., 2012). Both studies found that training combined with MVF resulted in an increased amount of bilateral performance gains when compared to more standard visual feedback (i.e., “normal” vision of the hand undertaking the task), a finding that might partially be related to the dominance of visual perception over proprioceptive feedback (Brass et al., 2001; Edwards et al., 2003; Touzalin-Chretien et al., 2010). Considering older adults’ greater reliance on visual feedback during the acquisition and performance of motor tasks (Swinnen et al., 2010; Swinnen et al., 1998; Voelcker-Rehage, 2008), investigating whether providing augmented visual feedback (i.e., MVF) might also enhance CLT in the ageing population, appears an important and promising line of investigation.

2.6. Summary

This chapter provided the reader with the underlying knowledge and framework required to understand the rationale behind the current research project, which is aimed at further investigating the role of visual feedback in CSE and performance gains in younger and older adults. The reader was familiarised with the phenomenon of CLT and introduced to the most up-to-date views in regard to potential adaptation sites and underlying mechanisms mediating the phenomenon. Moreover, studies that have suggested CLT to be augmented by the provision of MVF in younger people were presented; a potentially promising approach to be used within the context of ageing. However, highlighting unresolved questions regarding the approach of mirror training within rehabilitation, the chapter emphasised why more research is warranted to consolidate previous findings and further investigate the exact mechanisms underlying this promising research tool along with its use to augment CLT in younger and older adults.

CHAPTER 3: STUDY 1

Visual feedback-related changes in ipsilateral cortical excitability during unimanual movement: implications for mirror therapy.

Reissig, P., Garry, M. I., Summers, J. J., & Hinder, M. R. (2014). Visual feedback-related changes in ipsilateral cortical excitability during unimanual movement: implications for mirror therapy. *Neuropsychological Rehabilitation, 24*(6), 936-957.

3.1. Abstract

Provision of a mirror image of a hand undertaking a motor task (i.e., mirror therapy) elicits behavioural improvements in the inactive hand. A greater understanding of the neural mechanisms underpinning this phenomenon is required to maximise its potential for rehabilitation across the lifespan e.g., following hemiparesis or unilateral weakness. Young and older participants performed unilateral finger abductions with no visual feedback, with feedback of the active or passive hands, or with a mirror image of the active hand. Transcranial magnetic stimulation was used to assess feedback-related changes in two neurophysiological measures thought to be involved in inter-manual transfer of skill, namely corticospinal excitability (CSE) and intracortical inhibition (SICI) in the passive hemisphere. Task performance led to CSE increases, accompanied by decreases of SICI, in all visual feedback conditions relative to rest. However, the changes due to mirror feedback were not significantly different to those observed in the other (more standard) visual conditions. Accordingly, the unimanual motor action itself, rather than modifications in visual feedback, appears more instrumental in driving changes in CSE and SICI. Therefore, changes in CSE and SICI are unlikely to underpin the behavioural benefits of mirror therapy. We discuss implications for rehabilitation and directions of future research.

3.2. Introduction

Mirror therapy (MT) is a psychophysiological technique, first established by Ramachandran and Rogers-Ramachandran (1996), as a visual illusion to alleviate phantom limb pain, a condition in which a person experiences the painful sensation of an amputated or missing limb. MT involves placement of a mirror in a person's midsagittal plane, thus superimposing a mirror image of one limb over the (obscured) contralateral limb. Since its conception, research focusing on improving (behavioural and/or subjective) outcome measures such as motor performance or pain reduction (Thieme et al., 2012) has provided substantial support for the use of MT in stroke rehabilitation (for an overview see Altschuler et al., 1999; Thieme et al., 2012; Yavuzer et al., 2008) and in the treatment of chronic regional pain syndrome (CRPS) (McCabe et al., 2003; for an overview see Ramachandran & Altschuler, 2009). Considerably less research, however, has focussed on investigating the neural mechanisms which underlie the manifested behavioural changes elicited via MT.

Contrary to behavioural MT studies which, despite their relatively small sample sizes, appear to show consistent beneficial effects of mirror training with regard to improved outcome measures (Altschuler et al., 1999; Chan et al., 2007; Ramachandran et al., 1995) the consensus regarding the neural underpinnings of mirror training is much less certain. A possible reason for this might be the wide array of imaging and brain stimulation techniques employed by the relatively few studies that have attempted to elucidate the neural mechanisms underlying MT, such as functional magnetic resonance imaging (fMRI) (Shinoura et al., 2008), transcranial magnetic stimulation (TMS) (Carson & Ruddy, 2012; Fukumura et al., 2007; Funase et al., 2007; Garry et al., 2005; Lappchen et al., 2012; Nojima et al.,

2012), and electroencephalography (EEG) (Touzalin-Chretien & Dufour, 2008). Methodological differences in regard to task complexity (i.e., simple versus complex motor tasks), outcome measures used (i.e., tests of motor behavioural improvement in both the limb undertaking the task and the contralateral quiescent limb), and the nature of the visual feedback (e.g., vision of both limbs versus solely vision of the mirror feedback) provided during the experiments may also contribute to the absence of a consensus regarding the neural mechanisms that mediate the improved behavioural outcomes. A more precise understanding of the neural basis of the mirror feedback phenomena (e.g., changes in corticospinal excitability as well as specific changes in intra- and inter-cortical circuits) is, however, crucial if MT is to be adopted more widely as a rehabilitation and clinical tool for a number of distinct clinical populations (e.g., stroke sufferers, as well as those suffering from chronic pain or unilateral muscle weakness to name just a few). As such, more neurophysiological studies on MT are required.

To investigate the potential neurophysiological mechanisms underpinning MT, previous studies have predominantly examined changes within the hemisphere ipsilateral to a moving limb during a unilateral motor task (Fukumura et al., 2007; Funase et al., 2007; Garry et al., 2005; Nojima et al., 2012) on the assumption that excitability increases in this hemisphere are related to performance gains in the untrained limb (e.g., Carroll et al., 2008; also see Garry et al., 2005; Hinder et al., 2010a, 2010b; Lee et al., 2010). In 2005 Garry et al. asked participants to perform a continuous (rhythmic) unimanual finger abduction-adduction task, while provided with different types of visual feedback (Garry et al., 2005). TMS was applied over the M1 ipsilateral to the moving hand. Results demonstrated a generalised excitability increase in the ipsilateral (inactive) hemisphere during unilateral movements relative

to a resting condition (where both limbs were quiescent) regardless of the nature of the visual feedback provided. More importantly however, a Mirror Vision condition (in which participants watched a mirror image of the moving hand) led to larger excitability increases in ipsilateral primary motor areas than the other visual feedback conditions (such as viewing the active hand, the passive hand or fixating on a centrally aligned mark). The authors suggested that this additional excitability increase in the mirror feedback condition may underlie the behavioural benefits of mirror feedback reported in previous studies, such as performance gains in the untrained limb following viewing a mirror image of the trained limb during a motor task (Altschuler et al., 1999). Several subsequent studies (Lappchen et al., 2012; Nojima et al., 2012; Shinoura et al., 2008; Touzalin-Chretien & Dufour, 2008) have formed conclusions that are consistent with Garry et al.' (2005) original findings; however other studies (Fukumura et al., 2007) have only partially supported Garry et al. or have provided quite differing conclusions (Carson & Ruddy, 2012). For instance, in 2007 Fukumura et al. conducted a study in which participants performed a left hand wrist movement under different task conditions in which they were provided with mirror feedback of their active hand and/or imagined movement of the passive hand. Only when the motor task was undertaken in combination with motor imagery did mirror feedback lead to larger excitability increases (as measured by TMS-induced motor evoked potentials during task execution) in the ipsilateral hemisphere compared to those observed in the non-mirror conditions. Using a similar motor task, Carson and Ruddy (2012) asked participants to perform a unimanual left hand wrist movement under different experimental conditions (No Vision, Mirror Vision, Passive Vision). Although Mirror Vision induced ipsilateral excitability increases, these changes were not significantly greater than in the

condition in which participants did not see either hand. Moreover, it was found that vision of the passive hand lead to a significantly *lower* excitability increase in the hemisphere ipsilateral to the working hand when compared to Mirror Vision or No Vision conditions. Taken together, Fukumura et al. (2007) and Carson and Ruddy (2012) results show that modifications in visual feedback during a motor task affect the magnitude of the increase in corticospinal excitability in the hemisphere ipsilateral to a unimanual movement. However, contrary to the earlier findings by Garry et al. (2005), their results do not support the notion that MT confers a greater increase in ipsilateral corticospinal excitability than other feedback conditions. As such, these studies would argue against the supposition that the behavioural benefits of MT are a result of a greater increase in ipsilateral corticospinal excitability (Garry et al., 2005).

The apparently disparate conclusions with regard to the efficacy of mirror feedback in promoting increases in excitability of the ipsilateral hemisphere may, at least in part, stem from a lack of formal definition as to what should be concluded to represent a beneficial neurophysiological effect. In this regard, based on the assumption that mirror feedback results in improved performance in an untrained limb due to its effect on corticospinal excitability in the ipsilateral hemisphere, we propose that for mirror feedback to represent a valuable therapeutic intervention it *must* promote changes in corticospinal excitability that significantly *outweigh* changes that are elicited when the motor task is undertaken in the usual manner. Specifically, because one generally focuses on the hand undertaking a motor task (e.g., reaching and grasping a cup of tea, turning a key in a lock), we propose that mirror vision must elicit excitability changes (in the ipsilateral cortex) *above and beyond* those which occur when directly viewing the active hand. It is apparent that

the vast majority of the aforementioned studies failed to apply this criterion when forming their conclusions with regard to the efficacy of mirror feedback; indeed a number of studies (Carson & Ruddy, 2012; Fukumura et al., 2007; Nojima et al., 2012) did not investigate the effects of vision of the active hand to permit the suggested comparison to be made retrospectively.

If we re-appraise the results of the aforementioned studies in regard to the definition of beneficial neurophysiological changes following MT, it is apparent that of those which did employ a condition that focussed on the active hand (Funase et al., 2007; Garry et al., 2005; Tominaga et al., 2011), all failed to demonstrate mirror vision-elicited changes in corticospinal excitability that were significantly larger than those manifested in the standard visual condition (i.e., vision of the active hand). In only one study (Garry et al., 2005) did this comparison approach significance (p = .069), and, supported through a large effect size value (d = 0.91), may have failed to reach statistical significance as a result of the small sample size employed in that study. Therefore, while there appears to be some evidence to suggest mirror vision may promote ipsilateral cortex excitability, when assessed under our formalised definition, more research is required to further investigate this hypothesis.

Even though the (behavioural) effects of mirror feedback have been investigated in different clinical populations (Altschuler et al., 1999; McCabe et al., 2003; Yavuzer et al., 2008), to our knowledge no studies have specifically addressed MT in the context of ageing. This is surprising given that ageing incontrovertibly leads to functional and structural changes in the brain that not only affect behavioural aspects of motor performance (Seidler et al., 2010), but also neural control mechanisms underpinning movement performance (Fujiyama et al., 2009; Hinder et al., 2012;

Hinder et al., 2011; Talelli et al., 2008; Ward, 2006). Furthermore, older adults are more reliant on visual control and feedback to permit accurate motor performance (Swinnen et al., 1998; Voelcker-Rehage, 2008), and use more visual strategies while learning and performing motor tasks (Swinnen et al., 2010). Thus, a pertinent question is whether older adults exhibit greater responses (in terms of ipsilateral corticospinal excitability) to mirror vision when provided with it during the performance of a motor task. If this was found to be the case then MT would have the potential to be applied in rehabilitation programmes that are specifically tailored to aiding recovery in older people after injury. It could assist in regaining the loss of independence due to unilateral impaired motor functions not only after stroke (Thieme et al., 2012), but also after fall-related injuries; two of the major concerns which can adversely affect motor control in older people.

The current experiment had two main aims: Firstly, we wished to pursue the question of whether (as suggested by Garry et al.'s 2005 finding), mirror feedback promotes ipsilateral M1 facilitation compared to the more standard feedback conditions; secondly we aimed to determine the effects of MT in older populations where visual effects could be expected to be more profound. Because of the purported role of SICI in motor learning and transfer (Lappchen et al., 2012; Perez & Cohen, 2008), and because of age-related changes in SICI (Hinder et al., 2012; Peinemann, Lehner, Conrad, & Siebner, 2001) we also investigated intracortical inhibition in the active, and the inactive, hemisphere. The current experiment was undertaken following the study design of Garry et al.' (2005), and an *a-priori* power analysis revealed that a total 12 subjects would be required to detect an effect comparable to that reported in Garry et al. (d = 0.91). Considering we were also interested in

whether older adults exhibit greater responses to mirror vision (i.e., the addition of an extra variable) our study was conducted with two groups of 12.

3.2. Methods

3.2.1. Participants

Twelve younger (mean age = 24.6 years, *SD* = 4.7, eight men and four women) and 12 older (mean age = 70.3 years, *SD* = 5.5, three men and nine women) adults participated in the experiment. All participants were right-handed (Edinburgh Handedness Inventory, (Oldfield, 1971)), had normal or corrected-to-normal vision and were screened for contraindications to transcranial magnetic stimulation (TMS) (see Appendix). Additionally, a medical history questionnaire revealed that they were free from any known neuromuscular disorders and did not have a history of neurological illnesses that might affect neurophysiological measures (as assessed by TMS). The experimental procedures were approved by, and carried out in accordance with, local ethical guidelines laid down by the Tasmanian Human Research Ethics Committee Network, and conformed to the declaration of Helsinki. Prior to beginning the experiment participants asked any questions regarding techniques and procedures and, when they were happy, signed an informed consent form. Subjects either received course credit, or $20 reimbursement for their research participation.

3.2.2. Movement task

Participants were seated in a height adjustable chair with their forearms resting on the table and their palms facing down. Subjects were asked to perform discrete unilateral index finger abduction movements, carried out with either the left, or right hand (instructed by the experimenter) and consisting of both a dynamic movement phase and a tonic (isometric) contraction phase. The hand undertaking the task is

referred to as the ‘active’ hand, while the contralateral hand, which remained quiescent throughout the movement trials, is referred to as the ‘inactive’ hand. The initial part of the movements (index finger abduction) was performed against the resistance of a rubber band, which was put around the index finger and the middle finger of both hands (i.e., one rubber band on each side). Participants then maintained an isometric force against this resistance (i.e., the stretched elastic band) before relaxing such that their index finger returned back to the start position without resistance or effort (index finger adduction). The tension in the band was adjusted individually for each participant such that they could undertake the task “without excessive finger force and without finding repetitive movements fatiguing”. An auditory metronome (0.5 Hz, 500 ms tone duration) was used to pace participants’ movements. One complete abduction-adduction cycle was performed on each beat of the metronome (duration of one complete cycle = 2000 ms). Participants were asked to synchronise their finger abduction with the onset of the metronome beat and to (tonically) maintain the index finger abducted for the duration of the tone. The start and end points of the movement were indicated by dots on the table, individually adjusted to ensure reasonably large movement amplitudes for each participant (i.e., participants were asked to move to the end of their biomechanical range of motion during the abduction phase, while the rest of the hand was kept still and relaxed).

During the period in which participants undertook the movements they were provided with different types of visual feedback. In accordance with Garry et al. (2005) there were a total of five different visual experimental conditions: Active Vision, Passive Vision, Central Vision, Mirror Vision and Baseline. In the Active and Passive Vision conditions, participants visually fixated on the active or inactive hand, respectively,

while vision of the opposite (unattended) hand was occluded with a wooden box. In the Central Vision condition, participants looked straight ahead fixating a centrally aligned marker in the wall and vision of both hands was prevented by covering them with two boxes. In the Mirror Vision condition, a mirror was placed vertically in the midsagittal plane and participants viewed a mirror reflection of their active hand. Direct vision of the inactive hand was not possible due to the positioning of the mirror; however, the mirror image of the active hand appeared superimposed on top of the obscured position of the inactive hand. A custom-built screen, situated in the coronal plane between participants' upper body and their active hand, further prevented a direct view of the active hand (see Figure 3.1.). In all four of these (active task) conditions, participants undertook the unilateral motor task. In the fifth condition (Baseline condition) *both* hands remained quiescent, participants looked straight ahead and vision of both hands was prevented by covering each of them with a box.

Figure 3.1. Experimental set up for the Mirror Vision condition

A mirror was placed vertically in the midsagittal plane and participants viewed a mirror reflection of their active hand, with the mirror image of the active hand appearing superimposed on top of the obscured position of the inactive hand. A custom-built screen, situated in the coronal plane between participants' upper body and their active hand, further prevented a direct view of the active hand.

In each active task, participants performed two blocks of 30 trials (one trial = one movement) with each hand (for a total of 20 blocks, each of one minute duration). The order of hands and visual conditions was counterbalanced between subjects, with the exception of the Baseline condition, which was always performed prior to (Baseline 1) and following (Baseline 2) the four active conditions for each hand. Participants received one familiarisation trial with the Active Vision condition prior to the main experiment, and were allowed rest breaks between blocks to minimise

possible fatigue effects. The experiment lasted approximately two hours, which included set-up time and familiarisation with the experimental task.

3.2.3. Electromyographic recordings

Bilateral electromyographic (EMG) recordings were obtained from the left and right first dorsal interosseus (FDI) muscles, the primary agonists for the finger abduction task. Participants' skin was prepared with a lightly abrasive gel and cleaned with an alcohol wipe before attaching Ag/AgCl electrodes (Meditrace 130, Tyco Healthcare, Mansfield, MA) in a belly-tendon montage. EMG signals were amplified (X1000) and a notch filter (50 Hz) was applied prior to sampling using a 16-bit AD system (Power 1401, CED Limited, Cambridge, UK) and collected data was stored on a computer for subsequent offline analysis. EMG recordings enabled us to monitor task accuracy (i.e., movement synchrony with the tone of the metronome beat in the active hand) as well as quiescence in the inactive hand during task execution.

3.2.4. Transcranial magnetic stimulation

TMS was used to investigate corticospinal excitability and short interval intracortical inhibition (SICI) of the motor pathways from the inactive left and right motor cortices (lM1 and rM1) during right and left hand movements, respectively (i.e., we measured excitability of projections from M1 ipsilateral to the movement). TMS was delivered by two Magstim 200 magnetic stimulators (Magstim Company, UK) connected by a Bistim unit and a figure of eight coil (70 mm diameter).

The position over the M1 that consistently induced the largest motor evoked potentials (MEPs) in the contralateral muscle of interest was defined as the motor

hotspot. It was determined by placing the coil over the approximate location of the representation of the left and right FDI within M1 (~ 5 cm lateral and 2 cm anterior to Cz) and subsequently moving the coil around in small steps to different scalp positions to identify the location in which suprathreshold stimulation consistently produced the largest MEPs in the target muscle. The exact location orientation of the coil (with posterior-to-anterior-induced current in the cortex, i.e., coils at ~45° to the midline and in a plane tangential to the scalp surface) was then marked on the scalp and TMS intensity was reduced in 2% increments until the lowest TMS intensity was identified that elicited at least three out of five MEPs ≥ 50 µV (Garry et al., 2005; Hinder et al., 2011; Rossini et al., 1994). This intensity was deemed to be resting motor threshold (RMT). During the experiment alternating single-pulse and paired-pulse stimulation were delivered to the M1 of the inactive hemisphere. Single-pulse magnetic stimulation at suprathreshold (130% RMT) was applied to assess corticospinal excitability of the projections from the inactive hemisphere. Paired-pulse magnetic stimulation was applied to assess intracortical inhibitory processes (SICI). SICI was measured according to a paired- pulse paradigm by applying a subthreshold conditioning stimulus before a suprathreshold test stimulus (130% RMT) with an interstimulus interval (ISI) of 3 ms (Kujirai et al., 1993). The conditioning pulse was initially set to 70% of RMT, but subsequently adjusted to ensure that the elicited MEPs were suppressed by approximately 50%. TMS was delivered within every fifth movement cycle, 250 ms after the onset of the metronome beat during the isometric phase of the finger abduction. We recorded 12 MEPs per block (six single-pulse and six paired-pulse MEPs) and conducted two blocks per hand and visual condition, thereby collecting 24 MEPs for each condition (12 single-pulse and 12 paired-pulse MEPs).

3.2.5. Data and statistical analysis

We firstly visually inspected all trials to ensure that EMG activity in the FDI of the active hand was present prior to and at the time point of TMS delivery (i.e., synchrony of muscle activation with the onset of the metronome beat), indicating that a tonic contraction was present during the delivery of each TMS pulse. Trials with poorly timed, or absent, FDI activation (i.e., asynchrony of movement (AOM)) were discarded from further analysis. Following removal of these trials we calculated root-mean-squared (rms) EMG in the *inactive* hand ($rmsEMG_{inactive}$) in the period 115 - 15 ms prior to each TMS pulse. Additional trials were excluded from further analysis if $rmsEMG_{inactive}$ exceeded 0.025 mV. In the remaining trials, rms EMG of the *active* hand ($rmsEMG_{active}$) was calculated in the same time window, while peak-to-peak MEP amplitudes elicited in the FDI of the inactive hand by TMS were calculated in the 50 ms period commencing 15 ms after TMS delivery. Single-pulse MEP amplitudes were averaged and normalised to the MEPs obtained during the Baseline conditions in each hand on a participant-by-participant basis. Normalised (n) MEPs were subsequently log transformed (referred to as Ln nMEP) to reduce skewness that is otherwise associated with normalised (i.e., ratio) data (Hinder et al., 2010a; Sinclair & Hammond, 2008). Paired-pulse MEP amplitudes were averaged for each vision condition and divided by the corresponding single-pulse MEP amplitudes to calculate a SICI ratio for all five vision conditions (referred to as SICI). Accordingly, SICI < 1 indicates inhibition is present, with lower SICI indicating greater inhibition. SICI was log transformed and normalised to SICI obtained during the Baseline conditions (referred to as Ln nSICI) in each hand on a participant-by-participant basis. Positive Ln nSICI represent a decrease in inhibition while negative values indicate an increase in inhibition, relative to Baseline.

The number of rejected trials (due to voluntary activity in the inactive hand or asynchrony of movements in the active hand) was compared using independent sample t-tests to compare rejection rates across participant groups. Additional independent t-tests were performed to further compare differences in raw single-pulse MEP values as well as raw SICI at Baseline across participant groups. To compare $rmsEMG_{active}$, Ln nMEP and Ln nSICI we used repeated measures analysis of variance (RM ANOVAs) with hand (left, right) and vision condition (Active Vision, Mirror Vision, Central Vision, Passive Vision, Baseline) as within-subject factors and age (younger, older) as a between-subjects factor. Analysis of $rmsEMG_{active}$ investigated whether there were statistically significant differences in the strength of the voluntary contraction in the four visual conditions in which an active contraction was required. Analysis of Ln nMEP and Ln nSICI was aimed at investigating variations in corticospinal excitability and intracortical inhibitory processes in the inactive hemisphere as a function of visual feedback and age. The alpha level was set to 0.05 (with a Greenhouse-Geisser's degree of adjustment for violated assumed sphericity) and post-hoc pairwise comparisons examined all significant interactions using the Sidak adjustment. To aid the interpretation of the tests of significance partial eta-squared and Cohen's *d* are also presented and interpreted as a measure of effect size with cut-offs ≥ 0.2 small, ≥ 0.5 medium, ≥ 0.8 large for Cohen's *d* and ≥ 0.01 small, ≥ 0.06 medium, and ≥ 0.14 large for partial eta-squared (Cohen, 1988).

3.3. Results

All results are presented as means (*M*) ± standard deviations (*SD*), and 95% confidence intervals [CI]. Analysis did not reveal statistically significant differences between 100% RMT for participants of the younger group (46.3 ± 8.8, [42.4, 50.2]) and the older group (46.3 ± 8.5, [42.8, 49.9]) ($p = 1.0$).

3.3.1. Rejection rates

The average rate of discarded trials (over all participants in both groups) was 5.95% (5.74% due to increased EMG activity, 0.21% due to AOM). Independent *t*-tests compared the rejection rates due to high rmsEMG in the inactive hand and asynchrony of movement (AOM) in the active hand between participant groups. Participants of both groups did not differ significantly in their ability to synchronise their movements with the onset of the metronome beat during the four active conditions (AOM in % for younger group: $M = 0.15 \pm 0.16$, [0.05, 0.24], AOM in % for older group: $M = 0.28 \pm 0.28$, [0.12, 0.44]), $t(24) = 1.44$, $p = .167$, $d = 0.57$. Older subjects, however, showed a significantly higher rejection rate due to high rmsEMG in the inactive hand prior to the TMS pulse ($M = 8.6 \pm 8.2$, [3.0, 13.4]), when compared to participants of the younger group ($M = 2.9 \pm 4.0$, [1.5, 6.6]), $t(24) = 2.17$, $p = .045$, $d = 0.89$.

Since the strength of contraction in the active hand is able to influence the size of MEP amplitudes in the inactive hand (Liepert et al., 2001; Muellbacher et al., 2000), we also analysed $rmsEMG_{active}$. This analysis showed significantly larger $rmsEMG_{active}$ in the left hand (0.476 mV) in comparison to $rmsEMG_{active}$ in the right hand (0.371 mV), $F(1,22) = 9.83$, $p = 0.005$, $\eta_p^2 = 0.309$. However, more importantly,

$rmsEMG_{active}$ did not differ significantly across the four active viewing conditions, $F(3,66) = 1.01$, $p = 0.396$, $\eta_\rho^2 = 0.044$. Except for a trend towards significance for the interaction between hand and age ($p = 0.057$, younger: left hand versus right hand: $d = 0.78$, older: left hand versus right hand $d = 0.15$), no other significant differences were found (p-values > 0.2).

3.3.2. MEP amplitudes

Raw MEP amplitudes for both groups did not differ significantly at Baseline (younger group: $M = 1.93 \pm 1.22$ mV, [1.24, 2.62], older group: $M = 1.45 \pm 0.93$ mV, [0.91, 1.97]) ($p = 0.471$, $d = 0.45$). Accordingly, normalised and log transformed MEP values (Ln nMEP) were subsequently analysed in two steps. An initial analysis revealed an absence of main effects and interactions in which age was a factor (age: $F(1,22) = 0.12$, $p = 0.733$, $\eta_\rho^2 = 0.005$; hand x age: $F(1,22) = 0.36$, $p = 0.552$, $\eta_\rho^2 = 0.016$; vision x age: $F(4,88) = 1.01$, $p = 0.409$, $\eta_\rho^2 = 0.044$; hand x vision x age: $F(4,88) = 1.83$, $p = 0.130$, $\eta_\rho^2 = 0.077$) (see Figure 3.2. for an overview of Ln nMEP for the four active vision conditions).

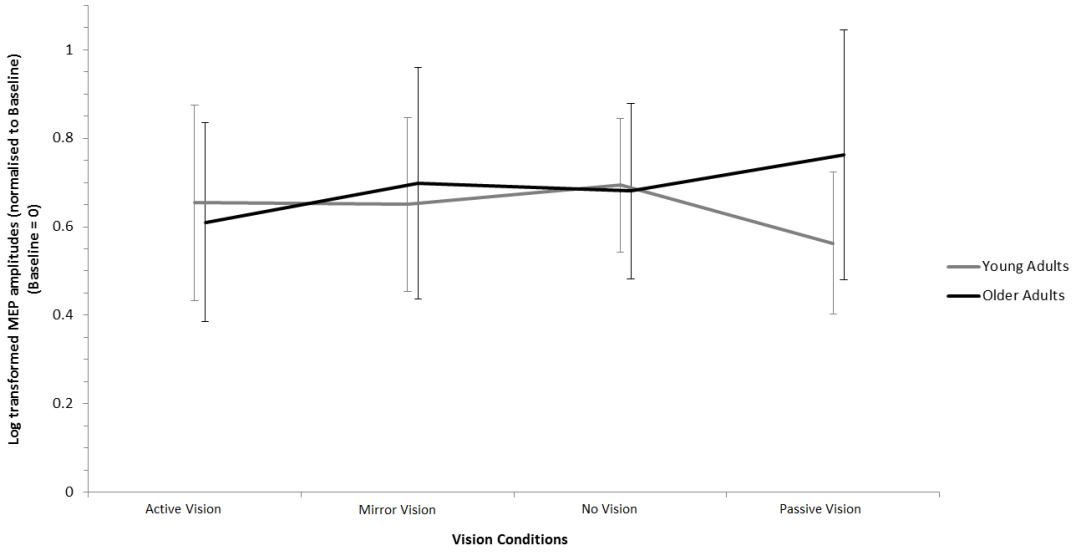

Figure 3.2. MEP amplitudes for the active conditions

Mean and 95% CI of log transformed and normalized MEP amplitudes (Ln nMEP) reported relative to Baseline MEP amplitudes (i.e., Baseline = 0 on the y-axis) for the four active vision conditions for younger and older groups.

Since that initial analysis did not reveal substantive effects involving the factor age, we collapsed the data across age and conducted a subsequent 2x5 RM ANOVA (N = 24). Analysis revealed a significant main effect of vision condition, $F(4,92) = 38.64$, $p < 0.001$, $\eta_\rho^2 = 0.627$. Post-hoc comparisons (Sidak) revealed that the MEP amplitudes in all four vision conditions were significantly enhanced compared to Baseline (all *p*-values < 0.001, all *d*-values > 2.0), indicating that unilateral activation increased ipsilateral corticospinal excitability. No other differences were found between the visual conditions (all *p*-values > 0.992, all *d*-values < 0.16, 95%CI). Furthermore, neither the main effect of hand, $F(1,23) = 0.74$, $p = 0.398$, $\eta_\rho^2 = 0.031$, nor the interaction of hand x vision, $F(4,92) = 1.17$, $p = 0.331$, $\eta_\rho^2 = 0.048$, were significant.

The analysis of Ln nSICI was conducted in the same manner as the Ln nMEP values. An initial t-test revealed that SICI at Baseline did not differ between participants of both groups (younger group: M = 0.52 ± 0.17, [0.49, 0.56], older group: M = 0.48 ± 0.17, [0.45, 0.52]) (p = 0.557, d = 0.24). As with Ln nMEP, the omnibus ANOVA for Ln nSICI failed to reveal any significant effects with age as a factor (age: $F(1,22) = 0.004$, $p = 0.941$, $\eta_\rho^2 < 0.001$; hand x age: $F(1,22) = 0.47$, $p = 0.499$, $\eta_\rho^2 = 0.021$; vision x age: $F(3,66) = 1.52$, $p = 0.217$, $\eta_\rho^2 = 0.064$; hand x vision x age: $F(3,66) = 1.53$, $p = 0.214$, $\eta_\rho^2 = 0.065$) (see Figure 3.3. for an overview of LN nSICI for all four active conditions).

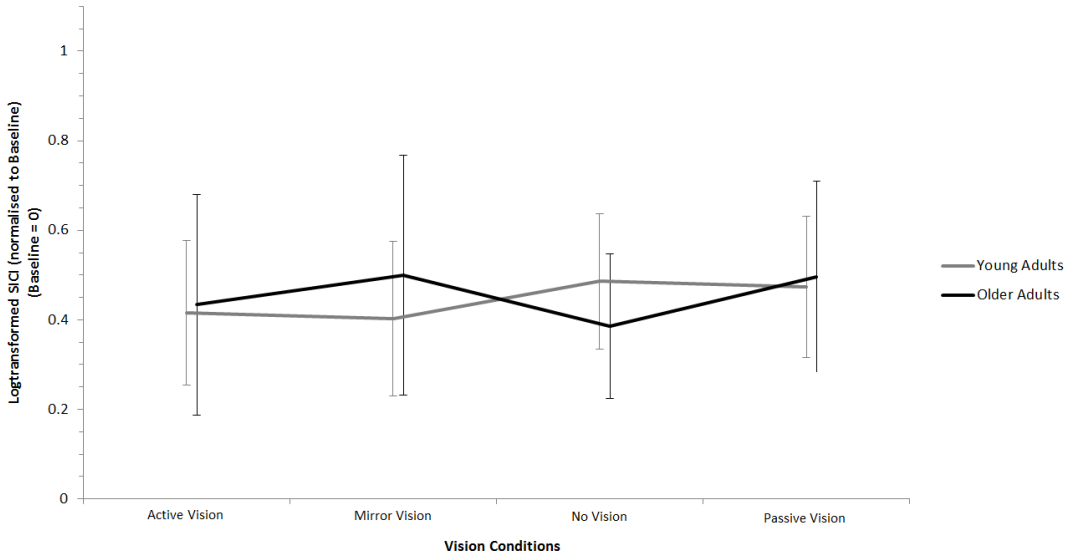

Figure 3.3. Short intracortical inhibition (SICI) for the active conditions

Mean and 95% CI of log transformed and normalized SICI (Ln nSICI) reported relative to Baseline SICI (i.e, Baseline = 0 on the y-axis) for the four active vision conditions for younger and older groups.

A subsequent 2x5 RM ANOVA (N = 24) conducted after collapsing across both age groups revealed a significant main effect of vision condition, $F(4,92) = 24.94$, $p <$

0.001, η_p^2 = 0.520. Post-hoc comparisons (Sidak) revealed that SICI in all four vision conditions was significantly enhanced compared to Baseline (all *p*-values < 0.001, all *d*-values > 1.6), indicating that unilateral activation resulted in a decreased intracortical inhibition in the ipsilateral hemisphere. No other differences were found between the visual conditions (all *p*-values > 0.911, all *d*-values < 0.18). Furthermore, neither the main effect of hand, $F(1,23) = 0.41$, $p = 0.527$, $\eta_p^2 = 0.018$, nor the interaction of hand x vision, $F(4,92) = 0.45$, $p = 0.773$, $\eta_p^2 = 0.019$, were significant.

3.4. Discussion

The current study investigated the extent to which different forms of visual feedback mediate the (well-described) increases in excitability of corticospinal projections to a passive limb during movements undertaken with the contralateral limb. In an attempt to provide insights into the neural mechanisms underlying the behavioural improvements resulting from MT (see Ramachandran & Altschuler, 2009; Thieme et al., 2012), a specific focus of this study was to assess whether mirror feedback of the moving limb led to more pronounced increases in ipsilateral corticospinal excitability compared to direct vision of the active limb, Furthermore, we were interested in investigating to what degree changes in ipsilateral intracortical inhibition (SICI) might be affected by provided visual feedback and how such modifications relate to induced changes in corticospinal excitability. Because MT is likely to be most beneficial in older populations (e.g., following stroke or immobilisation of a limb due to traumatic injuries or falls) we also aimed to determine whether ageing was associated with a change in the role of vision in mediating changes in corticospinal excitability and SICI ipsilateral to the moving limb.

Overall, we demonstrated that (relative to a condition when both hands were at rest) unimanual movements increase corticospinal excitability in the ipsilateral (e.g., passive) hemisphere when either no visual feedback of either hand, or feedback of the active hand, passive hand, or a mirror image of the active hand was provided. However, we failed to show any significant differences in the extent of these increases in corticospinal excitability *between* the different feedback conditions. Most notably, and in regard to our hypothesis that mirror visual feedback would provide additional excitability gains relative to the “ecological” or “natural” condition whereby

participants watched the active hand, the Mirror Vision condition conferred no additional excitability gains relative to the Active Vision condition. Accordingly, for the present task the unimanual motor action itself appears to be instrumental in modifying ipsilateral corticospinal excitability with the nature of the feedback provided while undertaking that task of little/less consequence.

The present results are not consistent with earlier studies that have noted the importance of specific types of visual feedback for facilitating the most potent changes in ipsilateral corticospinal excitability during motor tasks (Carson et al., 2005; Garry et al., 2005; Lappchen et al., 2012; Nojima et al., 2012), and for influencing the extent of performance gains in the untrained hand as a result of cross-limb transfer following a (unilateral) motor learning tasks (Lappchen et al., 2012; Nojima et al., 2012). It is apparent, however, that these studies did not specifically contrast neurophysiological or behavioural measures following provision of mirror visual feedback with those derived following direct visual feedback of the active hand. As alluded to previously, we propose that for mirror feedback to be concluded as being instrumental in driving performance gains in the untrained limb (e.g., with respect to cross-limb transfer) or in driving neural adaptation in the untrained/inactive cortex, the behavioural or neural adaptation following mirror visual feedback must be more pronounced than that observed following visual feedback of the active hand. Accordingly, while offering some insight into the mechanisms underlying MT, current research has not been able to offer definitive answers as to why MT subjectively appears to have such profound effects (see Ramachandran & Altschuler, 2009; Thieme et al., 2012).

Facilitation of corticospinal excitability in the passive hemisphere due to voluntary contraction of the ipsilateral limb has been shown in many previous studies (Aziz-Zadeh, Maeda, Zaidel, Mazziotta, & Iacoboni, 2002; Muellbacher et al., 2000; Perez & Cohen, 2008; Strafella & Paus, 2000) and has been proposed to occur due to crossed facilitation of neural pathways (Ruddy & Carson, 2013). There is evidence to suggest that the increases in ipsilateral corticospinal excitability are driven by decreases of intracortical mechanisms within the ipsilateral hemisphere (Muellbacher et al., 2000), as well as by an interaction between intracortical and transcallosal circuits (i.e., decrease of SICI and increase in IHI) (Perez & Cohen, 2008). The current results, which showed a decrease in SICI in the ipsilateral hemisphere in all four active conditions when compared to Baseline, do support the notion that increases in corticospinal excitability were driven, at least in part, by a decrease in SICI in the ipsilateral hemisphere.

It is apparent that, in the absence of volitional movement of either limb, profound effects on brain circuits and motor behaviour can also be evoked by movement/action observation (Aziz-Zadeh et al., 2002; Strafella & Paus, 2000). Specifically, action observation (AO) effects have been demonstrated to cause short term changes in corticospinal excitability (i.e., during movement observation; see Fadiga et al., 1995), as well as long term changes in motor cortical functions (i.e., formation of a motor memory as assessed by changes in motor representation; see Stefan et al., 2005), and have often been associated with the existence of an action observation/action execution matching system in the human brain (Touzalin-Chretien & Dufour, 2008). Even though originally associated with observation of another individual performing a task (Aziz-Zadeh et al., 2002; Strafella & Paus, 2000), AO-like effects have also been proposed to be responsible for the beneficial behavioural

effects of mirror feedback during *self-execution* of a motor task (Garry et al., 2005; Lappchen et al., 2012; Nojima et al., 2012). Garry et al. (2005) previously argued that putative therapeutic effects, such as an improved motor performance measured as range of motion, speed and accuracy in hemiparetic patients, which have been reported in previous behavioural-focused MT research (Altschuler et al., 1999; Yavuzer et al., 2008), may be caused by an *interaction* of voluntary unimanual movements and AO effects. The current study allowed us to go some way in determining which of these factors is most instrumental in driving the observed excitability change in the ipsilateral hemisphere that has previously been reported in MT-based research (Garry et al., 2005; Lappchen et al., 2012; Nojima et al., 2012). Based on our results, which showed a significant facilitation in MEP amplitudes in *all* active conditions (all $p < 0.001$, all d-values > 2.0) compared to the Baseline condition, but no significant difference in the extent of the facilitation observed in the Mirror Vision and Active Vision conditions ($p = 1.0$, d-value = 0.16), the unimanual movement appears to play a more important role in modifying corticospinal excitability in the passive hemisphere than observing the unimanual action through provided (mirror) visual feedback (i.e., AO). Moreover, although our results showed the ipsilateral corticospinal excitability increase was accompanied by a decrease in SICI, we did not find significant differences in the extent of this decrease in inhibition as a consequence of the various feedback conditions between the Mirror / Active Vision condition and the Central Vision condition (excitability: all $p > 0.992$, $d < 0.16$; SICI: all $p > 0.911$, all $d < 0.18$). The absence of differences in ipsilateral corticospinal excitability increase and in the decrease SICI between the Active Vision and the Mirror Vision condition indicates that the variation in visual feedback provided during task performance, asking participants to either focus on their

contralateral limb (Mirror Vision) or their ipsilateral limb (Active Vision), does not appear to be the underlying / driving factor with regards to previously reported MT-related behavioural improvements (see Ramachandran & Altschuler, 2009; Thieme et al., 2012). Our finding is in agreement with Cowles et al. (Cowles et al., 2013), who showed that AO effects provided little additional benefit on top of conventional practice effects in the recovery early after stroke. However, caution must be applied when relating results of an acute stroke population to the present study conducted in healthy young and older adults. In summary, our results suggest mirror feedback – when considered to represent AO-like effects - cannot be regarded as the *most influential* factor with regard to enhancing ipsilateral corticospinal excitability or decreasing ipsilateral SICI during MT, at least with respect to the particular finger abduction movement task employed in our study.

Considering the large 95% CI around the small effect size (*d*-value = 0.16) obtained when considering differences in ipsilateral excitability between the Mirror Vision and the Active Vision Conditions (95% CI [-0.46, 0.67]), the nature of the provided feedback may *potentially* still play a role in inducing corticospinal excitability changes than can be assumed solely on the basis of the current non-significant findings. That is MT and mirror feedback, when used within a therapeutic setting, may therefore still be capable of underpinning increases in ipsilateral corticospinal excitability that have previously been associated with MT-based behavioural improvements. However, given the apparently small effect size observed herein, in order to confidently conclude whether the nature of the visual feedback plays an important role in mediating changes in ipsilateral corticospinal excitability, studies with large sample sizes appear necessary. For example, a sample size of approximately 90 participants would be required if the true Cohen's *d* effect size was 0.3.

The current study also aimed to determine whether healthy ageing is associated with a change in the efficacy of visual feedback in mediating changes in corticospinal excitability ipsilateral to the moving limb. As indicated previously, healthy ageing results in numerous changes in the brain that have been shown to influence behavioural aspects of motor performance, such as coordination, speed, gait and balance (for an overview see Seidler et al., 2010). Furthermore, ageing is associated with changes in the neural control mechanisms that underpin movement performance (Fujiyama et al., 2009; Hinder et al., 2012; Hinder et al., 2011), and more specifically can result in changes in the efficient ability to modulate intrahemispheric (Hortobagyi & DeVita, 2006; Peinemann et al., 2001) and interhemispheric inhibitory mechanisms (Talelli et al., 2008), with the latter mechanism being related to the reduced integrity of the corpus callosum in later life (Hoy et al., 2004). Reduced callosal inhibition is linked to changes that affect the behavioural aspects of motor performance in older populations, and is (amongst other indicators) expressed as an increase in bilateral activation during the execution of unilateral motor tasks (when compared with younger adults). Evidence for this stronger ipsilateral corticomotor output during unimanual movement tasks (i.e., less lateralised task-related activation in primary and non-primary motor areas) in the elderly has been provided within imaging studies (Ward & Frackowiak, 2003), as well as within behavioural studies measuring output at the level of the muscles, i.e., mirror activity (Hinder et al., 2011). Considering the greater propensity for mirror activity in older people, together with their greater reliance on visual feedback and control to permit accurate motor performance (Swinnen et al., 1998; Voelcker-Rehage, 2008), we hypothesised that older people would be predisposed (compared to younger adults) to manifest changes in ipsilateral excitability on the basis of

changes in visual feedback. However, this hypothesis was not supported by the current experiment; that is, older adults did not show any greater degree of excitability change in response to mirror vision than did younger adults. Furthermore, our results did not show significant feedback-related differences in the modulation of intracortical inhibitory processes (examined by way of SICI) between younger and older participants. Both age groups exhibited SICI that was similar at rest, together with a commensurate decrease in SICI in the inactive hemisphere (consistent with Hinder et al., 2011) due the ipsilateral unimanual movement (regardless of the provided visual feedback). Because previous studies have failed to address the neural mechanisms of MT in aged populations, this result adds new, albeit unexpected, knowledge to the application of mirror feedback-based approaches in the older population.

It is possible that the present motor task was not complex enough to elicit or reveal age-related variations in neural responses to the provided visual feedback, at least with respect to overall changes in corticospinal excitability or SICI. Indeed, age-related performance differences are more visible in complex than in simple tasks (Dykiert, Der, Starr, & Deary, 2012; Fujiyama, Hinder, Garry, & Summers, 2013; Voelcker-Rehage, 2008), whereas performance is comparable for younger and older adults in more simple task (Breitenstein, Daum, & Schugens, 1996). It is therefore possible that the current task was not demanding enough to force older adults to engage in strategies to utilise visual feedback to ensure task accuracy. Alternatively, vision may not have had an effect on age-related variation in excitability in the current motor task due to the fact that provision of visual feedback was not required for an accurate, successful task execution. It is possible that a more goal-directed task, representative of an fundamental everyday action (e.g., a reaching movement

to a specific point in which feedback would have helped with the performance), would have caused the effects of the different vision conditions to be more obvious, especially within the older participants, when considering their demonstrated greater reliance on visual feedback and control to permit accurate motor performance (Swinnen et al., 1998; Voelcker-Rehage, 2008).

Previous research has shown that older adults exhibit additional activation in other motor-related regions (e.g., premotor and prefrontal cortex) during movement tasks in comparison with younger adults (Heuninckx, Wenderoth, Debaere, Peeters, & Swinnen, 2005; Hutchinson et al., 2002; Mattay et al., 2002; for an overview see Seidler et al., 2010). Accordingly, it is possible that changes may have occurred upstream of M1 and thus remained undetected in the current experiments. Indeed, recent work from our lab has demonstrated age-related changes in PMd and M1 interhemispheric connectivity during a simple reaction time (RT) task to be associated with different levels of motor performance in older adults (Fujiyama, Hinder, & Summers, 2013; Hinder et al., 2012). In a simple RT task (i.e., left index finger abduction) Hinder et al. (2012) showed that modulation of LPMd-RM1 interaction early in the preparation period was associated with faster responses in a group of older, but not in younger, participants. In a subsequent study Fujiyama et al. (2013), used the same simple RT task and a disruptive (virtual lesion) TMS approach to demonstrate a causal role of the left PMd in preparing right hand movements. Considering this greater connectivity between lPMd-rM1 to be important in the planning and execution of ipsilateral movements in older adults, future studies examining changes within this network could be beneficial to further investigate the potential for mirror feedback-induced neurophysiological effects in younger and older populations (see Ruddy & Carson, 2013). It is conceivable that changes upstream of

M1 (between secondary or preparatory motor regions and primary motor cortex), or within several regions of the parietal or occipital cortex (Filimon, Nelson, Huang, & Sereno, 2009; Rossit, McAdam, McLean, Goodale, & Culham, 2013) may relate to the seemingly robust positive effects of MT at the behavioural level. An investigation of the corresponding networks may therefore be helpful to further uncover the neural mechanisms underlying the behavioural benefits of MT (see Ramachandran & Altschuler, 2009; Thieme et al., 2012).

Further evidence to suggest areas upstream of M1 may play a role in MT is the finding that AO activates PMv (Iacoboni et al., 1999) and enhances connections between PMv and M1 (Lago et al., 2010). That is interhemispheric and intrahemispheric connectivity in areas upstream of M1 may play an important role in MT. Furthermore, AO facilitation, even though mainly found in M1 (Aziz-Zadeh et al., 2002), has also been detected in premotor areas (Iacoboni et al., 1999; Rizzolatti, Fogassi, & Gallese, 2001). Therefore, assuming that cortico-cortical projections from the premotor cortex to M1 play a major role in mediating the influence of visual input on M1 excitability (Strafella & Paus, 2000), future TMS studies targeting those may be worthwhile.

A complementary approach to that discussed above may also involve combining TMS measurements with non-invasive brain stimulation techniques to up- or down-regulate specific motor regions of interest and investigating the impact upon the neural mechanisms (e.g., PMd-M1 connectivity) and how these are subsequently affected by alterations in visual feedback. Indeed, if net excitability (as investigated by traditional TMS measurements) is not the driving factor underlying the beneficial effect of mirror feedback (see Ramachandran & Altschuler, 2009; Thieme et al.,

2012), this approach could give further inside into the underlying neurological mechanisms of MT.

In summary, we have shown that, regardless of age, mirror feedback during a unilateral task does not promote greater changes in excitability and inhibition of ipsilateral corticospinal excitability than those elicited when provided with more standard forms of visual feedback. As such, the unimanual motor action itself appears more instrumental than the type of visual feedback in driving those manifested behavioural changes reported within MT. Future work is warranted to further determine the neural underpinnings of MT such that its clinical benefit can be maximised in younger and older populations.

3.5. Supplementary Material

The results of chapter 3, indicating a lack of specific enhanced MVF-induced changes in ipsilateral CSE, were not in agreement with Garry et al (2005). Firstly, given the ascertain made in the introduction that methodological differences between extant studies may have led to inconsistent findings across MVF literature, it is worth considering whether the discrepancy between the current findings and those of Garry et al. might have been due to very subtle task differences. Despite utilising similar index finger abduction tasks, Garry et al. (2005) employed a continuous (rhythmic) motion, whereas the current study used a more discrete task by altering the frequency of the metronome (from 0.5 Hz to 1 Hz) and requiring participants to maintain an isometric contraction at peak abduction for the duration of an audio tone. As the production of continuous movements has been shown to lead to a more focussed / isolated activation of primary motor areas compared to discrete movements (Schaal, Sternad, Osu, & Kawato, 2004), such a difference in the cortical origin of the motor programmes may conceivably have been responsible for more pronounced CSE change in the MVF condition in Garry's study.

3.5.1. The potential role of the type of motor task

To investigate the above mentioned postulation, and determine whether the type of motor task (i.e., continuous vs. discrete movements) could be considered a crucial factor in modulating MVF-induced CSE increases, a follow-up study (follow-up study 1), was conducted. To this end, using a continuous finger abduction task identical to that described by Garry et al., the influence of visual feedback on changes in ipsilateral CSE and SICI was assessed in 12 younger participants. Analysis revealed

significant differences in CSE increases between the visual feedback conditions, $F(3,33) = 3.46$, $p = .027$, $\eta_p^2 = .24$. Consistent with the results reported in chapter 3, a general movement-related increase in CSE was found (all $p < .001$). However, the results failed to show enhanced MVF-induced changes in CSE compared to changes elicited via direct vision of the active hand ($p = .569$). Interestingly, when participants were asked to focus on their inactive hand the results showed *less pronounced* CSE increases compared to when they focussed on their active hand ($p = .015$) or a mirror image of their moving hand superimposed over the inactive hand ($p = .052$) (see Figure 3.4.). Although movement-induced CSE increases in the ipsilateral hemisphere were accompanied by a decrease in inhibition, no significant differences in the degree of this decrease of SICI was found as a consequence of the various visual feedback conditions, $F(3,33) = 1.41$, $p = .258$, $\eta_p^2 = .11$.

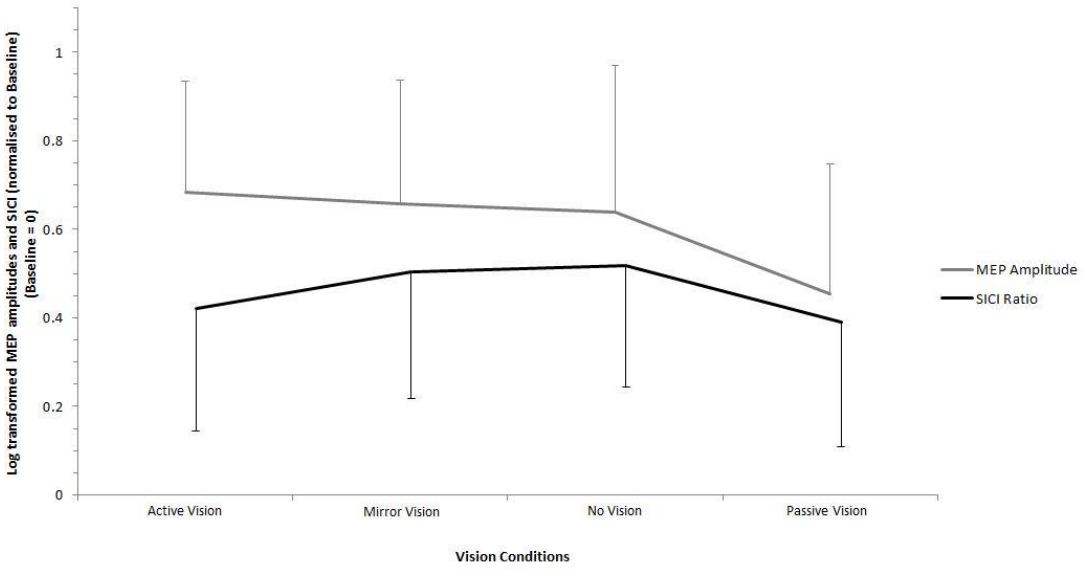

Figure 3.4. MEP amplitudes for the active conditions

Mean and 95% CI of log transformed and normalised MEP amplitudes (Ln nMEP) and SICI (Ln nSICI) reported relative to Baseline MEP amplitudes / SICI Ratios (i.e., Baseline - 0 on the y-axis) for the four active vision conditions for the 12 younger adults.

Based on the results of follow-up study 1, it seems that the influence of MVF on CSE changes in the ipsilateral hemisphere does not differ significantly depending on whether the motor task employed in the intervention is of continuous or discrete nature. The discrepancy between the findings in chapter 3 and previous work by Garry et al. (2005) is thus unlikely to be a result of subtle task differences between the two studies, but might be due to other less obvious differences in the methodology or the set-up of MVF-related research.

3.5.2. The potential role of long-lasting effects of different visual feedback conditions

One of the methodological or set-up-related characteristics (as alluded to above) which I was interested in exploring further to determine its potential impact on the findings reported in chapter 3 and the follow-up study 1, was based on a specific experimental finding in the study by Carson and Ruddy (2012). Close inspection of their work revealed that some of the reported visual feedback-related differences in ipsilateral CSE were apparent *at* the time of movement onset or even *before* onset occurred (see Figure 3 and Figure 4 in Carson & Ruddy, 2012). On the basis of the assumption that MVF induces greater CSE changes due to giving the impression the stationary hand is moving (at the time CSE is assessed using TMS), this finding is somewhat unexpected. It either indicates that the motor system regulates its excitability on the basis of knowledge (or predictions) of the forthcoming visual feedback, or that effects of visual feedback from a previous movement continue to modify CSE for a number of trials thereafter (such that CSE is modified prior to movement onset on subsequent movements).

Accordingly, it could be the case that the absence of differences between the different visual feedback modes in the previous experiments (chapter 3 and follow-up study 1) was due to the fact that participants were exposed to a number of visual feedback conditions within a single testing session. As such, some of the expected (theorised) effects of MVF could have been 'washed out' or diluted by previous exposure to the other visual conditions. Likewise, the effects of other visual conditions on CSE might have been overestimated had prior exposure to MVF occurred. Accordingly it is conceivable that, due to counterbalancing the order to which subjects were exposed to the different feedback conditions, over the whole experimental cohort no main effect of feedback on CSE was observed.

To investigate whether the results of chapter 3 and the follow-up study 1 were masked by long-lasting effects of different prior visual feedback conditions, four young participants took part in another experiment. In this follow-up study 2 participants performed a continuous unilateral movement (as described in follow-up study 1) under different visual feedback conditions in *two separate sessions* in which measurements of ipsilateral CSE and SICI were taken. Critically, only one manipulation of visual feedback was made in each session. In the first session, participants performed the motor task either focussing on a centrally aligned marker on the wall (i.e., No Vision condition) or on their moving hand (i.e., Active Vision condition), whereas in the second session they focused on a central fixation cross or were provided with a mirror image of their moving hand (i.e., Mirror Vision condition). Running two separate sessions also allowed for more blocks of movements to be performed in the same visual feedback condition and therefore enabled us to investigate whether MVF-induced changes in CSE became apparent following longer exposure. It was hypothesised that if prior exposure to MVF had long-lasting effects

on CSE (that could manifest as increases in CSE prior to movement onset in subsequent movements, as per Carson & Ruddy, 2012), MVF-related modification of CSE should become more robust following greater exposure to this mode of visual feedback. Results showed significant CSE increases in active task conditions (No Vision, Active Vision, Mirror Vision) relative to rest (Baseline), $F(2,6) = 18.48$, $p = .003$, $\eta_p^2 = .86$, and SICI decreases, $F(2,6) = 14.42$, $p = .005$, $\eta_p^2 = .83$. Consistent with the results of the study 1 (chapter 3) and the subsequent follow-up study 1 (see 6.2.1.1.), a general movement-related increase in CSE was found (all $p \leq .030$), which was accompanied by decreases in SICI (all $p \leq .036$). However, results again failed to demonstrate MVF-specific enhanced CSE increases (all $p = .083$) or SICI decreases ($p = .480$) compared to a condition in which participants focussed on the moving limb. Moreover, both conditions (i.e., Mirror Vision and Active Vision) did not elicit enhanced CSE increases / SICI decreases when compared to a condition in which participants were asked to focus on a centrally aligned marker (i.e., No Vision) (all $p \geq .146$). Figure 3.5. provides an overview of changes in CSE and SICI for the individual sessions.

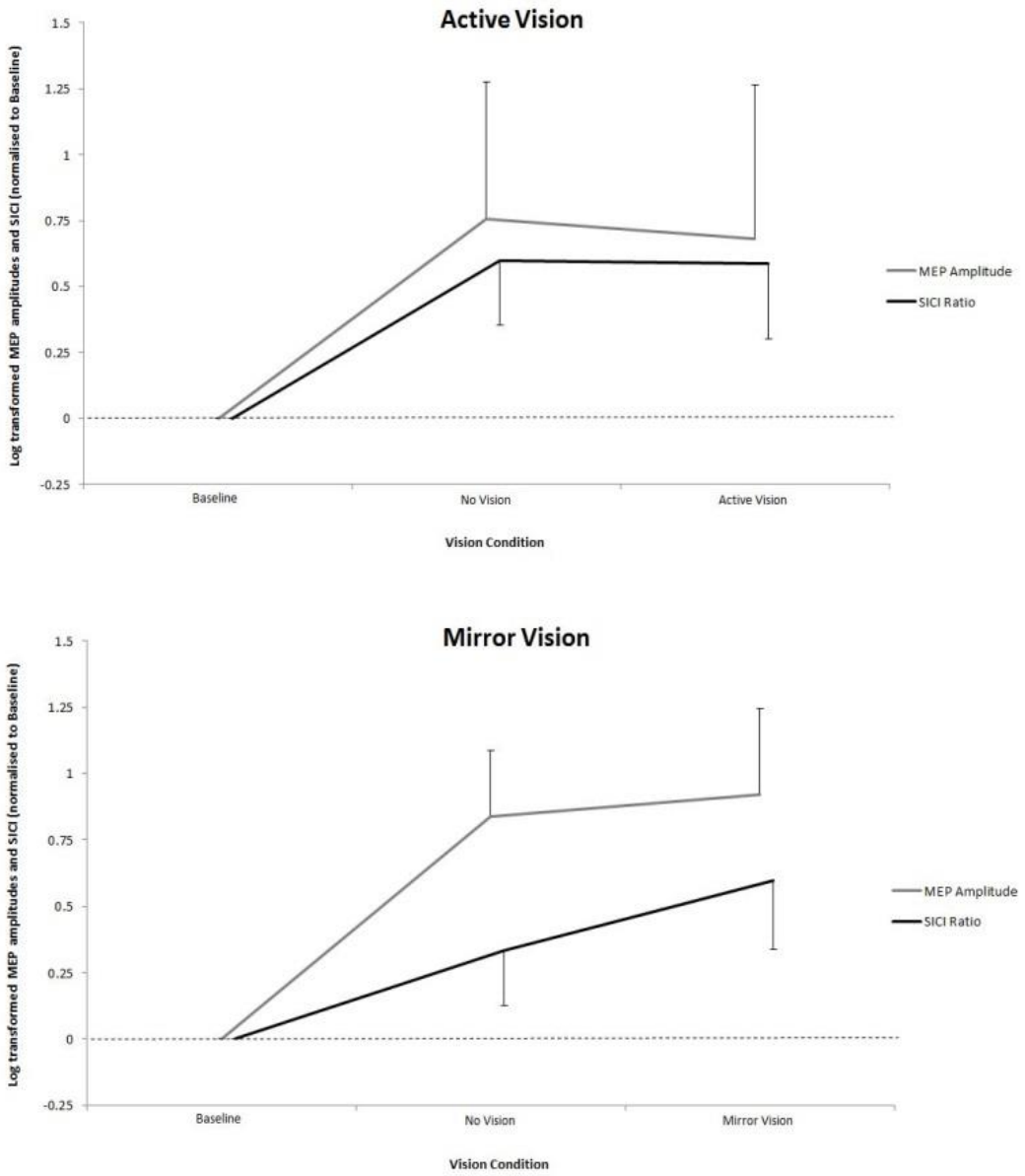

Figure 3.5. Changes in MEP amplitudes and short intracortical inhibition (SICI)

Mean and 95% CI of log transformed and normalised MEP amplitudes (Ln nMEP) and SICI (Ln nSICI) reported relative to Baseline MEP amplitudes / SICI Ratios (i.e., Baseline - 0 on the y-axis) for both sessions.

The findings of follow-up study 2 suggest that the lack of MVF-specific changes in CSE, as found in study 1 and the subsequent follow-up study 1, was not caused by participants previous exposure to different visual feedback conditions and an associated long-term effect of those conditions on CSE changes, respectively.

On the basis of the results from chapter 3, together with the two subsequent follow-up studies described above, the general conclusion that can be drawn is that MVF does not have a robust or observable effect on movement-related changes in CSE or SICI when compared to other forms of visual feedback. It may be the case that significant inter-participant variability limited the ability to observe robust effects in these studies, or that the magnitude of the effect has been over-estimated in previous research. Alternatively, it could be concluded that the present studies failed to support the notion that MVF modifies CSE or SICI during a repetitive motor action, suggesting another neurophysiological correlate may underlie well-documented MVF-effects with respect to behavioural outcomes.

3.5.3. The potential role of interhemispheric inhibition

In order to determine whether changes in CSE and SICI might have been the "wrong" measurements to assess MVF-induced changes on a neurophysiological level, follow-up study 3 focussed on another neurophysiological mechanism. Using the same motor task as used in follow-up studies 1 and 2, this study assessed changes in IHI from the active hemisphere onto the inactive hemisphere. Although two studies (Hamzei et al., 2012; Lappchen et al., 2012) recently failed to report MVF-induced changes in IHI, both studies contained a series of complex motor tasks, which differed from the simple continuous movements employed in the

present experiments. Indeed, a more recent study which appeared after the current research was completed (Avanzino et al., 2014) demonstrated a MVF-induced modulation of transcallosal communication (i.e., IHI) in a metronome-guided finger tapping task. This task is much more similar to that used in the previously-described experiments, and thus provides 'retrospective rationale' for the currently described follow-up study. Moreover, it should be noted that all three studies measured changes in IHI at rest (i.e., before and after the intervention). In accordance with a recent review by Ruddy and Carson (2013), changes measured at rest, however, do not necessarily have to relate to those occurring during the intervention. As such IHI at rest might therefore not be the most ideal way to assess transcallosal processes that underlie behavioural improvements resulting from MVF.

Six young participants were asked to take part in a study with an identical setup and procedure (in terms of two separate testing sessions) to follow-up study 2 to investigate task-related changes in CSE and IHI. IHI was measured via two individual coils delivering two suprathreshold TMS pulses (130% rMT), one to the M1 of each hemisphere. The initial CS was applied to the active hemisphere, and the subsequent TS was applied to the inactive hemisphere with an ISI of 10 ms (i.e., IHI 10) and 40 ms (i.e., IHI 40). Analysis revealed significant differences in CSE between the active task conditions and baseline, $F(2,10) = 18.74$, $p < .001$, $\eta_p^2 = .79$. As expected, and in agreement with the previous findings (chapter 3, follow-ups 1 and 2), results demonstrated a general movement-related increase in CSE (all $p \leq .013$), but failed to show statistically significant differences in CSE changes between conditions in which participants focussed on a centrally aligned marker and conditions during which participants either received MVF of their moving limb or focussed on their moving limb directly (all $p \geq .109$). Moreover, feedback-related

differences in transcallosal inhibition, assessed via IHI with 10 and 40 ms ISIs, did not reach statistical significance (all $p \geq .084$). Figure 3.6. provides an overview of changes in CSE and IHI for the individual sessions.

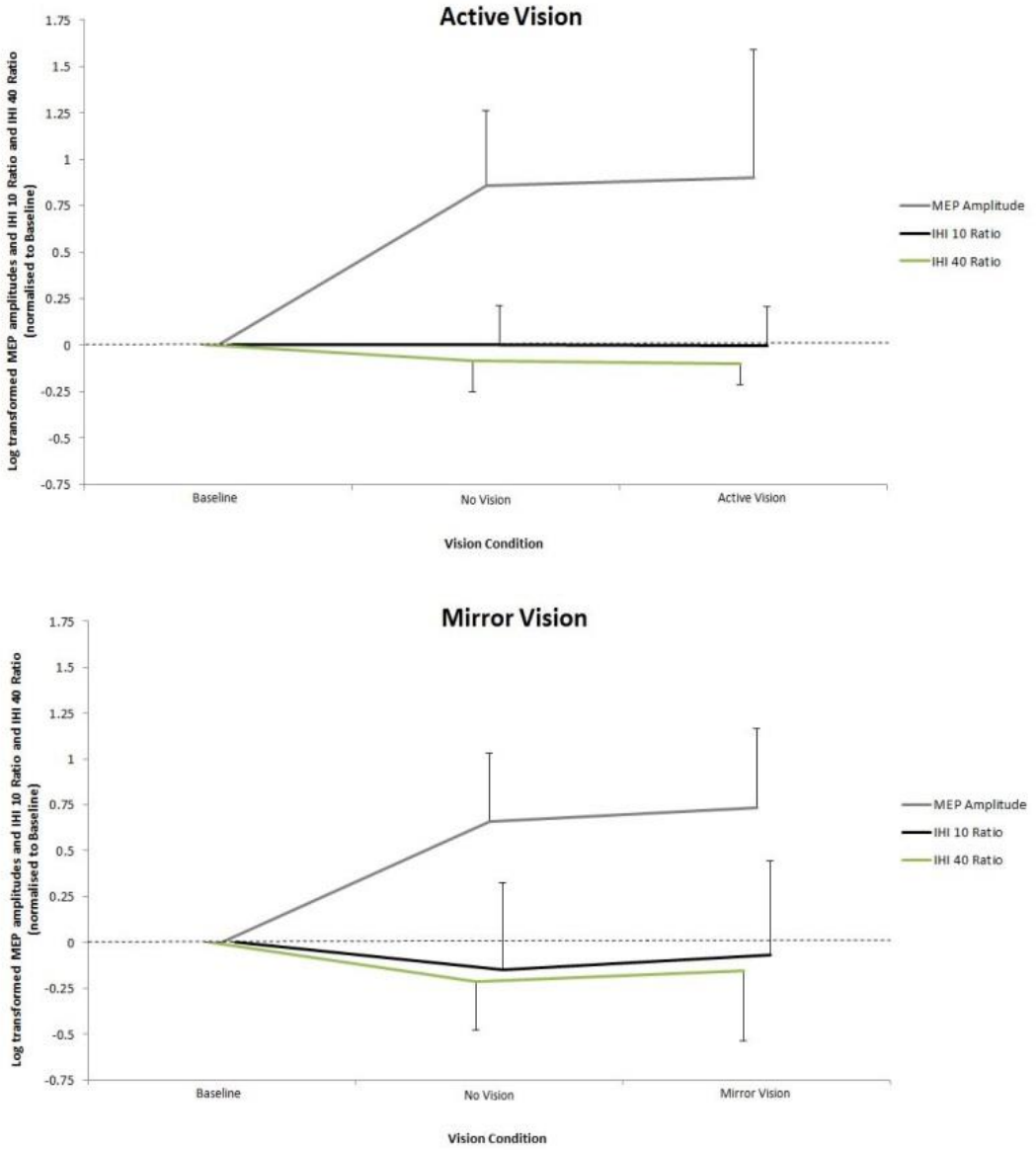

Figure 3.6. Changes in MEP amplitudes and interhemispheric inhibition (IHI)

Mean and 95% CI of log transformed and normalised MEP amplitudes (Ln nMEP) and IHI (Ln nIHI10 and Ln nIHI40) reported relative to Baseline MEP amplitudes / IHI Ratios (i.e., Baseline - 0 on the y-axis) for both sessions.

Based on the findings of follow-up study 3, it seems that IHI might also not be the neurophysiological mechanism underlying the previously reported MVF-induced behavioural improvements. In addition, the obtained results, again, did not support the previous reports of specific MVF-induced changes in CSE in the ipsilateral hemisphere.

CHAPTER 4: STUDY 2

The influence of mirror-visual feedback on training-induced motor performance gains in the untrained hand.

Reissig, P, Puri, R., Garry, M. I., Summers, J. J., & Hinder, M. R. (2015). The influence of mirror-visual feedback on training-induced motor performance gains in the untrained hand. *PLOS ONE. Scheduled publication date: 30th of October 2015.*

4.1. Abstract

The well-documented observation of bilateral performance gains following unilateral motor training, a phenomenon known as cross-limb transfer, has important implications for rehabilitation. It has recently been shown that provision of a mirror image of the active hand during unilateral motor training has the capacity to enhance the efficacy of this phenomenon when compared to training without augmented visual feedback (i.e., watching the *passive* hand), possibly via action observation effects (Nojima et al., 2012). The current experiment was designed to confirm whether mirror-visual feedback (MVF) during motor training can indeed elicit greater performance gains in the untrained hand compared to more standard visual feedback (i.e., watching the *active* hand). Furthermore, discussing the mechanisms underlying any such MVF-induced behavioural effects, we suggest that action observation and the cross-activation hypothesis may both play important roles in eliciting cross-limb transfer. Eighty participants (mean age = 27.5 years, 28 men) practiced a fast-as-possible two-ball rotation task with their dominant hand. During training, three different groups were provided with concurrent visual feedback of the active hand, inactive hand or a mirror image of the active hand with a fourth control group receiving no training. Pre- and post-training performance was measured in both hands. MVF did not increase the extent of training-induced performance changes in the untrained hand following unilateral training above and beyond those observed for other types of feedback. The data are consistent with the notion that cross-limb transfer, when combined with MVF, is mediated by cross-activation with action observation playing a less unique role than previously suggested. Further research is needed to replicate the current and previous studies to determine the clinical relevance and potential benefits of MVF for cases that, due to the severity of

impairment, rely on unilateral training programmes of the unaffected limb to drive changes in the contralateral affected limb.

4.2. Introduction

Mirror therapy is a psychophysiological technique used in the rehabilitation of individuals, who suffer from chronic regional pain syndrome or have experienced stroke or other forms of motor impairment, aiming to improve motor function or relieve pain. During mirror therapy a mirror is placed in an individual's midsagittal plane, which participants are subsequently asked to focus on. One limb is placed in the reflective side of the mirror, and its mirror image then superimposed over the contralateral limb that is hidden behind the mirror. Once the limb in front of the mirror is moved, a visual illusion of two synchronously moving limbs is created (see Figure 4.1.). Ramachandran and Rogers-Ramachandran (1996) were the first to employ mirror-visual feedback (MVF) to alleviate phantom limb pain. Since then, mirror therapy has also been demonstrated to be beneficial in stroke rehabilitation (Altschuler et al., 1999; Yavuzer et al., 2008) and in the treatment of chronic regional pain syndrome (McCabe et al., 2003). During bilateral movement therapy within a stroke rehabilitative environment, in which a participant aims to move both arms, MVF of the unimpaired arm is superimposed over the sensed position of the impaired (paretic) arm to give the impression that the impaired limb is moving as efficiently as the unimpaired limb. Such MVF has been previously shown to elicit behavioural improvements in the impaired limb that outweigh those which occur under normal unaltered visual conditions (i.e., direct vision of the impaired limb) (Altschuler et al., 1999; for an overview see Thieme et al., 2012; Yavuzer et al., 2008).

Most commonly used in conjunction with *bilateral* movement therapies within clinical settings (as described above), recent research has employed MVF in different types

of *unilateral* tasks (Lappchen et al., 2012; Nojima et al., 2012; Nojima, Oga, Fukuyama, Kawamata, & Mima, 2013) and suggests, at least for healthy populations, beneficial behavioural effects can occur. In this context, Nojima et al. (2012; 2013) recently asked participants to practice a fast-as-possible ball rotation task with their dominant hand while providing them with different types of visual feedback. Task performance was subsequently tested in both the trained and untrained hand. The authors found significantly better test performance in the untrained hand in the group that had received MVF during training, compared to the test performance in the untrained limb for a group that had focused on the passive hand during training. Moreover, the group receiving feedback of the passive hand also exhibited significantly impaired test performance in the untrained hand compared to a group that did not actually undertake any unilateral training but instead, passively watched a third person performing the motor task with the untrained hand. This latter result was interpreted to suggest that action observation (AO) effects – consisting of either watching one's own hand or a third person's – may drive a substantial proportion of the performance gains exhibited in the contralateral (untrained) limb (i.e., cross-limb transfer) following unilateral training under augmented visual feedback conditions (i.e., MVF).

Cross-limb transfer (CLT) has been recognised for more than a century, and has been demonstrated for various strength and skill acquisition tasks (for an overview see Carroll et al., 2006; Farthing, 2009; Hinder et al., 2013; Hinder et al., 2011; Lee et al., 2010). Despite a number of investigations (Carson & Ruddy, 2012; Garry et al., 2005; Reissig, Garry, Summers, & Hinder, 2014) the neural mechanisms underlying this phenomenon are not thoroughly understood. Different hypotheses have been put forward to describe the neural mechanisms of CLT and suggest that

either changes in the untrained hemisphere (i.e., cross-activation hypothesis) or changes in the trained hemisphere accessible by the untrained hemisphere (i.e., bilateral access hypothesis) occur in conjunction with behavioural gains in the trained limb, underpinning successful transfer of those behavioural gains (for more detail see Lee et al., 2010). However, as described above, given that Nojima et al. (2012) observed performance improvements in the untrained hand that were *not* contingent upon performance gains in the trained hand (nor any unilateral repeated practice at all), the idea that AO effects may be primarily responsible for CLT observed within mirror therapy was proposed. AO has been associated with the observation of *another individual* or of *oneself* performing a motor task (Aziz-Zadeh et al., 2002; Strafella & Paus, 2000) and is linked with an action observation/action execution matching system in the human brain which leads to the activation of similar brain areas when observing or executing the same movement (Touzalin-Chretien & Dufour, 2008).

In light of our ageing society, where stroke and mobility deficits induced due to fall-related injuries is becoming increasingly common, combining unilateral motor rehabilitation programs with mirror therapy is an appealing prospect. However, in order to improve the outcome of rehabilitative programs, it is important to shed further light on the underlying mechanisms of MVF-induced behavioural gains such that these programs can be designed to facilitate those factors that critically drive the transfer process. To this end, we expanded upon Nojima et al.' previous experiments (2012; 2013) by utilising the same motor task but including two additional visual conditions to tease apart the putative factors underlying crossed-effects in an untrained limb following motor training. Since it is most common to watch one's own hand when undertaking fine motor tasks to ensure accurate performance, we

employed a condition in which participants were provided with direct vision of the active hand during the training protocol (i.e., the most usual or ecologically valid visual feedback). In our previous studies of cross-limb transfer (Hinder et al., 2013; Hinder et al., 2011) this type of feedback has been associated with cross-limb transfer of behavioural gains, and would also be expected to drive transfer in a ball rotation task if this transfer was driven by gains in the trained limb (and associated cortical adaptations - Carroll et al., 2008; Lee et al., 2010). As we do not believe hand-specific AO-effects to be the sole underlying mechanism for the current movement task, we expected visual feedback of the active hand to also elicit transfer in the untrained hand, simply as a consequence of unilateral training. However, as it is possible that AO-effects might play an additional role in modulating CLT (Nojima et al., 2012; Nojima et al., 2013), we hypothesised the behavioural improvements in the untrained hand *may* be superior for the group that received MVF compared to the group that focussed on their active hand due to a combination of underlying AO and crossed-effects. Furthermore, we included a control condition, in which performance in the untrained hand was tested before and after a rest period of a commensurate amount of time to that taken for unilateral training in the other groups. We propose that such a condition would elucidate the extent to which performance improvements in the untrained limb may have occurred as a result of one-trial learning (i.e., conducting the test twice - Gates, 1917) as opposed to AO or crossed-effects occurring in conjunction with gains observed in the trained hand. Indeed, test-enhanced learning has been demonstrated in a variety of cognitive and behavioural tasks and its influence on Nojima et al.' paradigm cannot be assumed to be negligible.

4.3. Methods

4.3.1. Participants

Eighty members of the University of Tasmania community (mean age = 27.5 years, *SD* = 8.3 years, 28 men and 52 women; range 18 - 48 years) participated in a single session of 30 minutes duration. Six of the 80 adults (three men and three women) were left-handed (Edinburgh Handedness Inventory, (Oldfield, 1971), see Appendix), and all had normal or corrected-to-normal vision. The experimental procedure was approved by, and carried out in accordance with local ethical guidelines laid down by the Tasmanian Human Research Ethics Committee Network, and conformed to the Declaration of Helsinki. All participants signed an informed consent form prior to the experiment and received course credit for their research participation.

4.3.2. Movement task

Participants were seated in a height adjustable chair with their forearms rested on a table and their palms facing upwards. Participants performed a two-ball rotation task similar to the one previously utilized by Nojima et al. (2012, 2013). Specifically, they were asked to rotate two golf balls (43 mm diameter and 45 g) as quickly as possible in either a clockwise direction (with their right hand) or an anti-clockwise direction (with their left hand).

4.3.3. Experimental design

The study investigated the effects of a motor learning task with the dominant hand on subsequent motor performance of the non-dominant hand while the nature of visual feedback provided during motor learning was manipulated. Three groups of

participants practiced a fast-as-possible two-ball rotation task with their dominant hand while receiving different types of visual feedback. For a better pictorial representation of how the two balls were rotated within the palm, please refer to Figure 1A out of the Materials and Methods section of Nojima et al. (2015). Participants in the Active Vision (ACT: n = 20, females: 14, mean age = 28.3 years, *SD* = 8.2 years) and Passive Vision (PAS: n = 20, females: 15, mean age = 25.8 years, *SD* = 6.3 years) groups focused on the active (training) or inactive (non-training) hand, respectively, while vision of the opposite (unattended) hand was occluded with a custom built stand. For participants in the Mirror Vision group (MIR: n = 20, females: 11, mean age = 24.7 years, *SD* = 6.3 years), a mirror was placed vertically in the midsagittal plane and participants viewed a mirror reflection of their active hand. Direct vision of the inactive hand was obscured due to the positioning of the mirror; however, the mirror image of the active hand appeared superimposed on top of the obscured inactive hand. A custom-built stand, situated in the coronal plane between participants' upper body and their active hand, also prevented a direct view of the active hand. In these three groups, participants practiced 10 blocks of 30 seconds of ball rotation. Thirty seconds of rest was provided between each practice block to avoid fatigue and participants were regularly verbally encouraged to perform the task as fast as possible. Prior to, and following the training phase (total duration 10 min), participants performed the same task with their non-dominant hand for 30 seconds with similar instructions to perform the task as quickly as possible. Participants in the Control group (CON: n = 20, females: 12, mean age = 31.2 years, *SD* = 10.5 years) performed these two test blocks with their non-dominant hand, but rested between the blocks for a time period comparable to the training period in the other groups. Data of the first and last training block of the trained hand and the two

test blocks of the untrained hand was collected via video recordings and stored for subsequent analysis.

Figure 4.1. Experimental set up for the visual feedback conditions

Experimental set up for the visual feedback conditions in the three training groups: Mirror Vision (left), Passive Vision (middle), and Active Vision (right).

4.3.4. Data Reduction and statistical analysis

In accordance with previous work (Nojima et al., 2012) the study was designed as an independent groups design with repeated measures. To assess training-induced changes in performance of the dominant (trained) and non-dominant (untrained) hand, the video recordings were analysed and the number of ball rotations quantified in the pre- and post-test of the untrained hand ($pre_{untrained}$, $post_{untrained}$), along with the first and last block of motor learning for the trained hand ($pre_{trained}$, $post_{trained}$), thereafter referred to as pre- and post-performance in the trained and untrained hands. Post-performance was then normalized to pre-performance and subsequently natural log-transformed for both the trained [$n_{trained} = \ln(post_{trained} / pre_{trained})$] and untrained [$n_{untrained} = \ln(post_{untrained} / pre_{untrained})$] hands to avoid positive skewness that is commonly associated with normalized data.

Participants in the active training groups (ACT, PAS, MIR) who did not exhibit learning-induced performance improvements in the trained hand or did not exhibit transfer-induced performance improvements in the untrained hand were excluded from the analysis of trained and untrained hand performance. Firstly, to investigate potential differences in the trained and untrained hand at pre-test, we conducted one-way ANOVAs using $pre_{untrained}$, $pre_{trained}$. Subsequently, we investigated potential visual-feedback induced differences in the trained hand (dependent variable 1) by conducting a one-way ANOVA on $n_{trained}$ with groups (ACT, PAS, MIR) as a between-subject factor. Finally, to probe training-induced changes in performance of the untrained (non-dominant) hand (dependent variable 2), not only between the three training groups (i.e., ACT, PAS, MIR) but also relative to a CON group (i.e., participants that did not receive motor training with the dominant hand), we conducted a one-way ANOVA using $n_{untrained}$.

IBM SPSS Statistics 21 (Armonk, NY, USA) was used for all analyses with the *a priori* level of two-tailed significance set at 0.05. Both normalized trained and untrained variables were tested for normality using the Kolmogorov-Smirnov test and log transformed (ln) in the event of a violation of the assumption of normality prior to further analysis. *Post hoc t* tests were used to examine all significant main effects and multiple comparisons corrected using the False Discovery Rate (FDR) method (Benjamini & Hochberg, 1995). The FDR, a method used when conducting multiple comparisons, is designed to control the expected proportion of rejected null hypotheses that were incorrect rejections ("false discoveries"). The FDR method is less conservative compared to tests such as the Bonferroni correction, and therefore has greater power to detect true effects. Partial eta-squared (η_p^2) for ANOVA's, and

Cohen’s d for student’s t tests are provided as measures of effect size and used to aid in the interpretation of inferential statistics.

4.4. Results

All results are presented as means (*M*) ± standard deviations (*SD*), and 95% confidence intervals [CI]. There were no significant differences between the groups with regards to their age ($p > 0.05$). Table 1.1 shows the mean and SD for the raw number of ball rotations in each group for the trained and untrained hand at pre- and post-test.

	Mirror Vision		**Active Vision**		**Passive Vision**		**Control Group**	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Trained Hand	11.9 ± 5.1	15.7 ± 4.8	12.5 ± 3.7	15.8 ± 4.5	11.6 ± 3.0	15.3 ± 2.9	n.a.	
Untrained Hand	14.0 ± 5.3	16.7 ± 5.6	11.5 ± 4.8	13.5 ± 4.5	11.7 ± 4.8	13.9 ± 4.8	15.2 ± 6.0	16.2 ± 6.4

Table 1.1 Number of ball rotations in the trained and untrained hand:

Mean and SD representing the raw number of ball rotations in each group for the trained hand (N = 51) and the untrained hand (N = 59) at pre-and post-test.

4.4.1. Performance of the trained hand

An initial analysis conducted on the participants who satisfied the aforementioned inclusion criteria (n MIR = 16, n PAS = 18, n ACT = 17) did not reveal a significant difference raw performance in the trained hand at pre-test ($p = 0.794$). A subsequent one-way ANOVA also did not reveal a significant difference in raw performance in the trained hand at pre-test ($p = 0.794$). A subsequent one-way ANOVA also did not reveal a significant difference in motor-learning induced performance increases in

the trained hand, $F(2,51) = 0.66$, $p = 0.520$, $\eta_p^2 = 0.027$, between the three active groups (MIR = 0.32 ± 0.27, [0.22, 0.42]; PAS = 0.29 ± 0.15, [0.20, 0.39]; ACT = 0.24 ± 0.16, [0.15, 0.34]). Unsurprisingly however, regardless of feedback type, participants showed substantial improvements in the trained hand ($M = 0.28$) as revealed by a significant grand mean effect, $F(1,51) = 106.42$, $p < 0.001$, $\eta_p^2 = 0.689$. Figure 4.2. represents natural log-transformed normalized performance gains in the trained hand for each of the training groups.

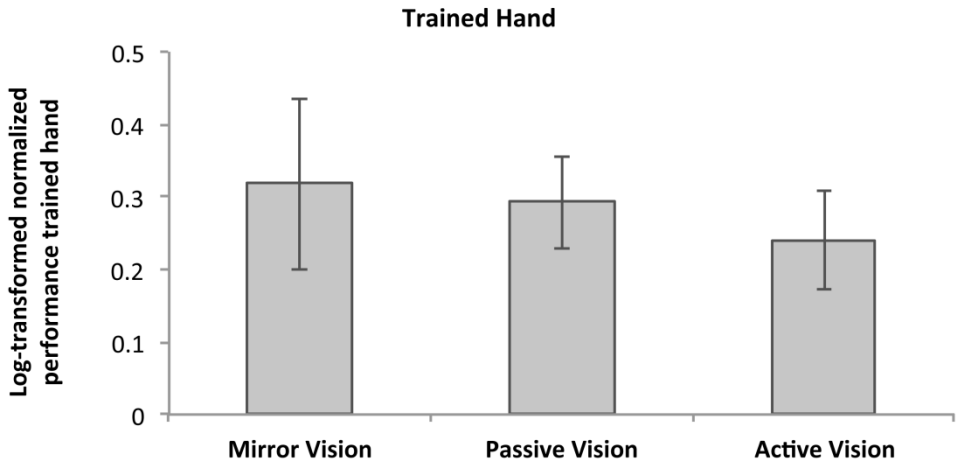

Figure 4.2. Performance changes trained hand (number of ball rotations)

Log-transformed normalized improvement in the trained hand: Averaged normalized performance changes (log-transformed) in the trained hand for each of the three training groups (N = 51). Error bars represent 95% CI.

4.4.2. Performance of the untrained hand

An initial analysis conducted on the participants who satisfied the aforementioned inclusion criteria (n MIR = 15, n PAS = 12, n ACT = 12, n CON = 20) revealed did not reveal a significant difference in raw performance in the untrained hand at pre-test (p = .178). A subsequent one-way ANOVA revealed significant differences in performance gains in the untrained hand between the groups, $F(3,59) = 6.06$, p = 0.001, η_p^2 = 0.248. Follow up FDR- corrected t tests revealed significantly smaller performance gains for participants in the control group (CON = 0.06 ± 0.10, [0.07, 0.12]) compared to all three training groups (MIR = 0.19 ± 0.11, [0.14, 0.25]; PAS = 0.19 ± 0.10, [0.13, 0.26]; ACT = 0.19 ± 0.14, [0.13, 0.25]) (all FDR-adjusted p's ≤ 0.028, all d's ≥ 1.093). Additionally, none of the training groups differed between each other with respect to the extent of gains in the untrained limb, indicating that the nature of the visual feedback provided during the motor learning task did not induce a statistically significant influence on performance gains in the untrained hand (all p's > 0.965). Figure 4.3. represents log-transformed normalized performance gains in the untrained hand for the training groups and the control group.

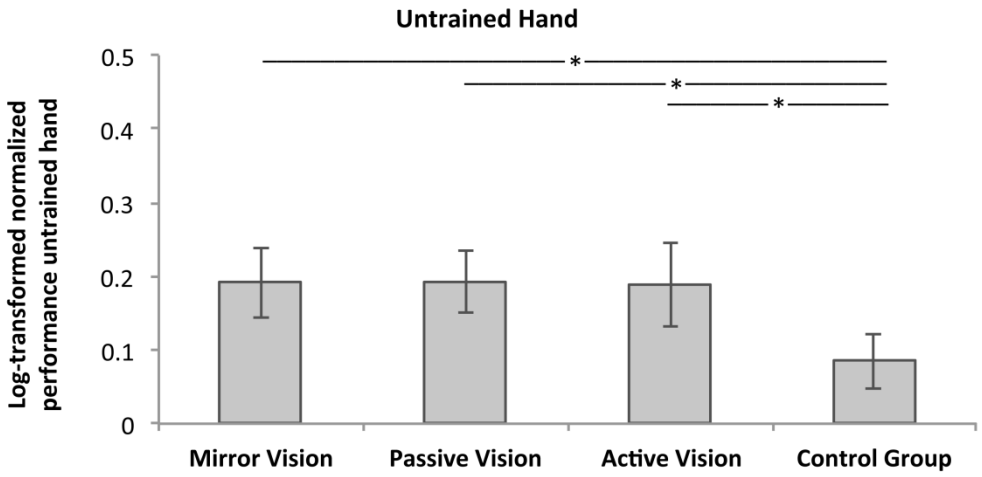

Figure 4.3. Performance changes untrained hand (number of ball rotations)

Log-transformed normalized improvement in the untrained hand: Averaged normalized performance changes (log-transformed) in the untrained hand for each of the three training groups and the control group (N = 59). Error bars represent 95% CI and * indicates FDR corrected $p < 0.05$.

A similar analysis conducted on those participants who *did not* exhibit training-induced effects in the trained hand (n = nine) revealed that normalized performance in the untrained hand (log-transformed) did not differ between the training groups (0.06 ± 0.16, [-0.02, 0.14]) and the CON group (0.06 ± 0.10, [0.07, 0.12]), $F(1,29) = 0.01$, $p = 0.940$, $\eta_p^2 < 0.001$.

4.5. Discussion

The current study aimed to determine whether MVF during unilateral motor training - in this case a fast-as-possible two-ball rotation - could result in significantly greater performance improvements in the untrained hand compared to conditions where participants focus on their active or passive hand during training, while shedding further light on the possible mechanisms mediating the transfer process. Building on the results of Nojima et al. (2012; 2013), which appear to indicate that MVF may be beneficial in facilitating performance gains exhibited in the untrained hand, we aimed to further investigate the main contributors to any such facilitation once any effects of single-trial learning are taken into account. Specifically, we examined the extent to which MVF facilitation can be attributed solely to action observation or whether other mechanisms such as (cortically-regulated) crossed-effects resulting from performance improvements in the trained limb may also be involved. Understanding these mechanisms and the extent of their influence on transfer is essential such that mirror therapy may be utilised more widely within rehabilitative and therapeutic settings to induce the greatest possible performance gains in the untrained limb, e.g., a paretic limb following stroke.

In line with earlier research examining cross-limb transfer across different strength and skill acquisition tasks (for an overview see Carroll et al., 2006; Farthing, 2009; Hinder et al., 2011; Lee et al., 2010), in the current study the degree of performance improvement after unilateral training (although seen in *both* the trained and untrained hands) was greater in the trained compared to the untrained hand. The finding that performance improvements in the trained hand did not vary between training groups indicates that any intermodal conflicts due to differences in visual feedback

(Deconinck et al., 2015; Ramachandran & Altschuler, 2009) were overcome such that performance gains were elicited. Moreover, these findings are consistent with the view that performance improvements in the trained limb are only partially manifested in the untrained limb (e.g., Hinder et al., 2013; Hinder et al., 2011; Lee et al., 2010). For those participants who exhibited performance gains in the trained hand, all feedback groups showed an increase in performance in the untrained hand (MIR: $n_{untrained}$ = 0.19, PAS: $n_{untrained}$ = 0.19, ACT: $n_{untrained}$ = 0.19) that was significantly greater than the improvement observed in the control group that had not undergone training ($n_{untrained}$ = 0.08) (p = 0.015). It can therefore be assumed that for the motor learning task employed in the current, as well as in previous studies (Nojima et al., 2012; Nojima et al., 2013; von Rein et al., 2015), the performance gains in the untrained hand were actually a consequence of prior motor training with the contralateral hand and did not simply occur as a consequence of participants performing the motor task with the untrained hand twice (i.e., pre- and post-test) – an effect previously demonstrated in a variety of cognitive and behavioural tasks (Morris, Bransford, & Franks, 1977; Roediger, 1990; Roediger & Karpicke, 2006).

With regards to the mechanisms mediating MVF-induced performance gains in the untrained limb, the current findings are not consistent with the idea of effector-specific AO effects as the primary underlying mechanism (Nojima et al., 2012). Rather, they are in support of the notion that training-induced improvements in the trained hand were imperative to induce performance improvements in the untrained limb. A number of lines of evidence support this view. Firstly, for all participants in *all* training groups, performance gains in the untrained hand - regardless of the nature of the visual feedback - were only apparent when training-evoked improvements in the trained limb were observed. That is, those participants who did not exhibit

learning with the trained hand following the training period exhibited no gains in the untrained hand (i.e., similar improvements to the control group) (p = 0.940). This suggests that performance improvements in the untrained limb are contingent upon performance gains in the trained limb, a notion which is not consistent with effector-specific AO but is in accordance with different theories underlying cross-limb transfer, such as the cross-activation hypothesis (Carroll et al., 2008; Lee et al., 2010). Secondly, if performance improvements in the untrained hand were predominantly a result of effector-specific or effector-congruent AO (i.e., facilitatory effects dependent on the congruency of the observed action), participants in our ACT group would not be expected to exhibit performance gains in the untrained hand, as observation occurred of the trained and not the untrained hand. However, they showed improvements in the untrained hand ($n_{untrained}$ = 0.19) that did not vary significantly relative to those participants who received MVF or focussed on their passive hand during the training regime (MIR: $n_{untrained}$ = 0.19, PAS: $n_{untrained}$ = 0.19) (all *p's* > 0.968) (Figure 4.3.). According to our results, MVF-induced behavioural improvements in the untrained hand can thus not solely be attributed to effector-specific AO effects, but are also mediated, at least in part, by crossed-effects which are contingent upon training-related adaptations and performance gains in the trained limb.

We propose that MVF-induced improvements in the untrained hand are mediated, at least partially, by mechanisms similar to those underlying cross-limb transfer following unilateral training programmes in the presence of standard modes of visual feedback (i.e., watching the active hand). This proposition differs from those suggested in a number of previous studies investigating MVF-induced transfer. Based on the finding of Nojima et al. (2012), two other studies (Hoff et al., 2015; von

Rein et al., 2015) recently argued that the neural mechanisms mediating MVF-related performance improvements in the untrained hand differed from those that mediate cross-limb transfer under more standard (veridical) visual feedback conditions. We believe that a number of behavioural and neurophysiological factors potentially contribute to the extent of cross-limb transfer exhibited after unilateral training using different visual feedback conditions. More research is thus needed to further investigate the exact contribution of those variables.

Contrary to previous reports by Nojima et al. (2012), we did not observe statistically significant differences in the degree of performance improvement in the untrained hand depending on the type of visual feedback provided during the task (Nojima et al., 2012; Nojima et al., 2013). The current findings are, however, consistent with results from a recent study (Reissig et al., 2014), in which we also proposed that a variation of visual feedback was unlikely to be the underlying driving factor behind previously reported mirror training-related behavioural improvements (Deconinck et al., 2015; Ramachandran & Altschuler, 2009; Thieme et al., 2012). Despite the purported association between increases in corticospinal excitability (as a measure of plasticity changes in the motor cortex) and motor learning processes, our previous study (Reissig et al., 2014) did not find increased corticospinal excitability facilitation in the ipsilateral motor cortex in the MVF condition when compared to more standard visual feedback conditions (i.e., watching the active or the passive hand). It was thus concluded that the unilateral execution of the movement itself represented the more important mechanism underpinning MVF-induced gains in the untrained hand, with AO-effects potentially being manifested concurrently to a lesser degree.

Alternatively, it is conceivable that the inconsistency in findings between the current study and Nojima et al. (2012) may reflect subtle differences in the experimental setup. Specifically, participants in the mirror group in the current study were *only* able to see the mirror image of the active hand (superimposed over the inactive hand), whereas in Nojima et al.' experiments (Nojima et al., 2012; Nojima et al., 2013) participants were permitted peripheral vision of the active hand as well as its mirror image. The cross-activation hypothesis of cross-limb transfer (Lee et al., 2010) is predicated upon the fact that unilateral tasks are associated with bilateral cortical activation, e.g., an increase in unilateral force leads to an excitability increase in the projections to the opposite limb (Perez & Cohen, 2008). Accordingly, allowing people to view both 'hands' (i.e., the active hand and the mirror image – as was the case in Nojima et al's studies) may have led to more pronounced changes in the M1 ipsilateral to the active hand and may subsequently have led to greater performance increases when compared to the MVF condition in the current study, where participants only saw a single limb. This view is also supported by previous research investigating the underlying neural mechanisms of MVF (Diers, Christmann, Koeppe, Ruf, & Flor, 2010; Fritzsch et al., 2014; Shinoura et al., 2008). Specifically, Fritzsch et al. (2014) argued that the production of additional ipsilateral activation in M1 from MVF might have been due to the availability of vision of the mirror *and* the active hand during task execution. However, as we did not attain any neurophysiological measures, nor test conditions in which one or both hands were visible, we are unable to determine whether this proposition holds true in the current experiment.

In considering a previous study by Nojima et al. (2015), which found behavioural improvements after AO to be dependent on and positively correlated to the degree of

kinaesthetic illusion elicited by the AO, it is conceivable that our MIR condition failed to induce a significant enough kinaesthetic illusion in the untrained hand such that this condition failed to elicit performance gains that were superior to those observed in the other feedback groups. One of the potential limitations to the conclusions drawn from the current study is the substantial inter-participant variability observed with respect to performance, both prior to, and following motor training, possibly suggesting a high degree of task complexity. Moreover, the degree of learning observed over the course of the training was very low (i.e., an increase of only two - three ball rotations within the 30 s period), which is likely to have resulted in any subtle differences in the extent of learning (in either the trained or transfer hand), eliciting due to changes in feedback, remaining undetected. An associated consideration is that, consistent with Nojima et al. (2012), we used the same sized balls for all participants. This may have made the task easier for some individuals, and harder for other, depending on whether the ball diameter was appropriate for their palm size. In addition, we used a set of balls that differed in terms of their size and weight (43 mm diameter and 45 g) compared to those used in previous studies (30 mm diameter and 10g weight, see Nojima et al., 2013; Nojima et al., 2013; von Rein et al., 2015). Such a difference may have accounted for the lower baseline performance in both the trained (mean = 15 rotations) and untrained (mean = 14 rotations) hand observed in the current study compared to the previously mentioned studies (Nojima (2012): approx. 21 rotations over a 30 second period; von Rein (2015): 43 rotations over a one minute period), further hindering sufficient performance gains and possible transfer. Finally, errors in coordination (i.e., ‘slips’ or ball drops), despite being corrected for quickly, could substantially affect the number of ball rotations achieved in the short 30 second test period resulting in large

variability. We propose that future studies employ an array of tasks of varying complexity (e.g., equipment size adjusted to hand size) that are sensitive enough to evaluate whether MVF is more effective for certain types of tasks. Moreover, we recommend evaluating motor performance throughout the entire training period (von Rein et al., 2015) as opposed to only assessing pre- and post-training measures, thus enabling more accurate conclusions to be drawn about participants' change in performance over time. In light of previous findings (Nojima, Koganemaru, Kawamata, Fukuyama, & Mima, 2015), we suggest assessment of the degree of kinaesthetic illusion elicited across the different feedback groups, as such illusory effects might be an important factor explaining and determining the success of MVF-based interventions. Finally, as it is possible that a 10-minute motor training protocol is insufficient time to detect reliable improvements in the untrained hand, we would suggest further research to employ a design using multiple training sessions of the same motor task.

In conclusion, the present study does not support previous suggestions that MVF has the potential to increase the extent of training-induced performance changes in the untrained hand following unilateral training above and beyond those observed for other types of feedback. Although somewhat speculative, our behavioural data are consistent with the notion that CLT effects are mediated, at least in part, by neural adaptations (Carroll et al., 2008) that occur in conjunction with behavioural gains in the trained limb, and AO, in contrast, appears to play not as significant a role as suggested by recent reports (Nojima et al., 2012). Further research is needed to replicate and expand upon the current and previous studies to determine clinical relevance, especially for cases in which rehabilitation using bilateral movement

therapies is not possible due to the severity of the impairment, and thus increasing the importance of unilateral training programs.

CHAPTER 5: STUDY 3

The role of mirror visual feedback and mirror muscle activity on cross-limb adaptations in young and older adults.

Reissig, P., Stöckel, T., Garry, M. I., Summers, J. J., & Hinder, M. R. (2015). The role of mirror visual feedback and mirror muscle activity on cross-limb adaptations in young and older adults. *Under review (Frontiers in Aging Neuroscience).*

5.1. Abstract

Cross-limb transfer (CLT) describes the observation of bilateral performance gains due to unilateral motor practice. Previous research has suggested that CLT may be reduced, or absent, in older adults, possibly due to age-related structural and functional brain changes. Based on research showing increases in CLT due to the provision of mirror visual feedback (MVF) during task execution in young adults, our study aimed to investigate whether MVF can facilitate CLT in older adults, who are known to be more reliant on visual feedback for accurate motor performance. Twenty-seven younger (mean age = 26.1 years, nine men) and 26 older participants (mean age = 69.6 years, 12 men) engaged in a short-term training regime (300 movements) involving a ballistic finger task using their dominant hand, while being provided with either visual feedback of their active limb, or a mirror reflection of their active limb (superimposed over the quiescent limb). Bilateral performance was examined before, during and following the training. Furthermore, we measured corticospinal excitability (using TMS) at these time points, and assessed muscle activity bilaterally during the task via EMG; these parameters were used to investigate the mechanisms mediating and predicting CLT. Training resulted in significant bilateral performance gains that did not differ as a result of age or visual feedback (all p's > 0.1). Training also elicited bilateral increases in corticospinal excitability (p < 0.05). For younger adults, CLT was significantly predicted by performance gains in the trained hand (β = 0.47), whereas for older adults it was significantly predicted by mirror activity in the untrained hand during training (β = 0.60). The present study suggests that older adults are capable of exhibiting CLT to a similar degree to younger adults. The prominent role of mirror activity in the untrained hand for CLT in older adults indicates that bilateral cortical activity during

unilateral motor tasks is a compensatory mechanism. In this particular task, MVF did not facilitate the extent of CLT.

5.2. Introduction

Unilateral training can induce performance increases in both the trained and untrained limb. Such bilateral performance gains are known as cross-limb transfer (CLT) or cross-education and have been shown in a variety of strength and skill acquisition tasks (for an overview see Carroll et al., 2006; Farthing, 2009; Ruddy & Carson, 2013). Recent work has suggested that ageing may be associated with a reduction in the extent to which CLT is manifested relative to that observed in younger adults (Graziadio, Nazarpour, Gretenkord, Jackson, & Eyre, 2015; Hinder et al., 2011; Parikh & Cole, 2013). However, the exact mechanisms underlying such an effect not yet completely understood.

Ageing is known to be associated with changes in motor performance (for an overview see Seidler et al., 2010), with increased bilateral activation (at the cortical or muscle level) during unilateral training observed across a number of tasks (Hinder et al., 2011; Mattay et al., 2002; Ward & Frackowiak, 2003). Such increases in mirror muscle activity in older adults are suggested to be caused by changes in the neural control mechanisms underpinning movement performance (Fujiyama et al., 2009; Hinder et al., 2012; Hinder et al., 2011), such as a decreased ability to modulate intra- and interhemispheric inhibitory mechanisms (for a review see Hoy et al., 2004; Talelli et al., 2008). As increased bilateral activation (at the cortical or muscle level) has been shown to be associated with enhanced motor performance in older adults (Bodwell et al., 2003; Mattay et al., 2002; Naccarato et al., 2006), Hinder et al. previously hypothesised that greater mirror activity (i.e., greater bilateral cortical activity measured via TMS) may promote greater transfer in older adults (Hinder et al., 2011). However, despite an increased level of mirror activity in the older adults,

Hinder et al. (2011) did not find a correlation between mirror activity and transfer and thus suggested the inability to regulate mirror activity may actually limit the transfer of motor skills in the advanced age.

As ageing is associated with an increased reliance on visual control and benefit from visual feedback for accurate motor performance (Swinnen et al., 1998; Voelcker-Rehage, 2008) we propose that another potential reason for the observed absence / decrease of transfer in previous studies (Hinder et al., 2011; Parikh & Cole, 2013) could have been the absence of a specific focus of attention on available visual feedback. Specifically, neither Hinder et al. (2011) nor Parikh and Cole (2013), who studied CLT in a group of younger and older people using the same motor task, specifically instructed their participants to maintain a focus of attention on visual feedback *during* task execution.

A special type of visual feedback is mirror-visual feedback (MVF), whereby a mirror image of one (usually active) limb is superimposed over the actual position of the other (usually inactive) limb by means of a mirror placed in a person's midsagittal plane. Mirror training (using MVF) was introduced by Ramachandran and Rogers-Ramachandran (1996) as a psychophysiological technique to alleviate phantom-limb pain. Although the exact underlying neural mechanisms of this phenomenon are incompletely understood (Carson & Ruddy, 2012; Garry et al., 2005; Ramachandran & Altschuler, 2009; Reissig et al., 2014), behavioral evidence indicates beneficial effects of mirror therapy within stroke rehabilitation (Altschuler et al., 1999; Yavuzer et al., 2008) or the treatment of chronic regional pain syndrome (McCabe et al., 2003). MVF could be viewed as a form of augmented visual feedback (Howatson, Zult, Farthing, Zijdewind, & Hortobagyi, 2013), which has been shown to increase

motor learning (Schmidt & Lee, 2011). More recently, MVF has also been demonstrated to be advantageous compared to more standard visual feedback (i.e., “normal” vision of the hand undertaking the task) when applied in a unilateral motor task, leading to enhanced CLT in younger people (Lappchen et al., 2012; Nojima et al., 2012). An approach to increase bilateral behavioural benefits via unilateral training appears particularly useful during rehabilitation of a limb following stroke or traumatic injury. This is particularly the case for older adults, as even short periods of immobilisation (e.g., splinting or casting of a limb due to a fall-related injury) have been shown to result in a significant loss of strength and consequently affect older adults’ functional ability to maintain an independent lifestyle. Intervention programmes should aim at minimising the loss of strength during periods of immobilisation, and ensure a quicker return to independent living. The utilisation of *unilateral* training paradigms that would result in bilateral performance changes are thus very appealing in an ageing population as they could be used during the period in which the affected limb is too weak to undertake physical training itself (or is indeed immobilised/cast due to fracture); as such this technique may maintain functional capacity by reducing the extent of functional losses during periods of immobility or weakness. The current study thus aimed to determine whether CLT may be enhanced by augmented sensory feedback (i.e., MVF) in older adults, who may have underlying deficits in the ability to exhibit CLT (Hinder et al., 2011; Parikh & Cole, 2013).

For the current experiment we employed a well-studied goal-directed ballistic finger movement task (i.e., aim to achieve peak acceleration) (Carroll et al., 2008; Hinder et al., 2011; Lee et al., 2010) that is known to share neural mechanisms with strength training paradigms (Selvanayagam et al., 2011) and moreover has been shown to

elicit a strong neural drive emerging from the contralateral primary motor cortex (M1). Activation of the motor cortex due to voluntary movements has previously been shown to facilitate cortical activation of the ipsilateral cortical areas, with an increasing force leading to increased bilateral activation (for an overview see Carroll et al., 2006; Dettmers et al., 1996; Muellbacher et al., 2000). Assuming CLT occurs substantially due to neural mechanisms the level of the cortex (Carroll et al., 2006; Lee et al., 2010) an activation of interhemispheric connections between left M1 – right M1 might be a crucial prerequisite for CLT to occur. We therefore propose that a combination of a goal-directed motor task that strongly engages the contralateral M1 combined together with mirror-visual feedback may lead to greater behavioural benefit (i.e., bilateral performance increase) in older adults when compared to younger adults.

We were also interested in investigating whether certain (behavioural/neurophysiological) parameters measured during the unilateral training period could explain performance increases observed in the untrained hand for both younger and older adults. Specifically, we were interested in the influence of two particular variables: Firstly, whether the extent of bilateral muscle activation exhibited during the acquisition of the present ballistic unilateral motor learning task was associated with the amount of subsequent transfer. This proposition was based on a previous study (Graziadio et al., 2015) showing greater transfer in older (compared to younger) adults in the feedforward control component of a motor learning task (previously associated with bilateral cortical activation only in older adults). In contrast, transfer was reduced in older adults relative to the younger adults in the feedback control component of the same task (previously associated with bilateral activation in both younger and older adults). Because our ballistic acceleration task

is driven by feedforward mechanisms (and thus associated with predominantly unilateral cortical activity in younger adults), we hypothesised that any bilateral activity in older adults may also facilitate cross-limb transfer. Secondly, we aimed to determine the extent to which the degree of performance improvements in the trained hand is associated with the subsequent degree of CLT in the untrained hand. Considering age-related changes with regard to behavioural and neural control of movements (Hinder et al., 2012; Hinder et al., 2011; Fujiyama et al., 2009) and interpreting previous findings of reduced CLT in older adults despite comparable improvements in the trained limb (Hinder et al., 2011; Parikh & Cole, 2013) as possible evidence for a change in the mechanisms underlying CLT with advancing age, we were interested in investigating whether there was a difference across age in the underlying factors predicting successful CLT.

5.3. Methods

5.3.1. Participants

Twenty-seven younger (mean age = 26.1 years, *SD* = 5.3, nine men) and 26 older (mean age = 69.6 years, *SD* = 5.6, 12 men) adults participated in the experiment. Fifty-one participants were right-handed and two left-handed (Edinburgh Handedness Inventory, (Oldfield, 1971)). All participants had normal or corrected-to-normal vision and were screened for contraindications to transcranial magnetic stimulation (TMS). Additionally, a medical history questionnaire revealed that they were free from any known neuromuscular disorders and did not have a history of neurological illnesses that might affect neurophysiological measures (as assessed by TMS). Finally, all participants were community dwelling with no known cognitive deficits. The experimental procedures were approved by, and carried out in accordance with local ethical guidelines laid down by the Tasmanian Human Research Ethics Committee Network, and conformed to the declaration of Helsinki. Prior to the beginning of the experiment participants asked any questions regarding techniques and procedures and, when they were satisfied, signed an informed consent form. Participants either received course credit, or were reimbursed $20.

5.3.2. Movement task

Participants were seated in a height adjustable chair with their forearms pronated and hands resting on a horizontal board to standardise hand position and isolate movements to their index finger. Participants were asked to perform unilateral ballistic abduction movements with their left and right index finger (see Hinder et al., 2013), while keeping the rest of the hand still. The aim of the task was to maximise

the horizontal peak acceleration of each movement, measured using an accelerometer (Dytran Insturments, Chatsworth, CA, USA/Endevco Corp. San Juan Capistrano, CA, USA) attached to the index finger with a plastic splint and tape.

5.3.3. Experimental design and procedure

Prior to motor training (pre-test), we measured corticospinal excitability and intracortical inhibition in both hemispheres. The neurophysiological testing was followed by a bilateral assessment of participants' motor performance (behavioural testing), consisting of 10 trials of the ballistic finger movement performed at 0.5 Hz paced by an auditory metronome. Subsequently, participants performed two blocks of 150 trials of the same task with their dominant hand and were provided with one of two forms of feedback during performance. Participants in the Active Vision (AV) group (younger group: 12, older group: 14) were asked to focus on their active hand, while vision of their inactive hand was occluded with a wooden box. For the Mirror Vision (MV) group (younger group: 15, older group: 12), a mirror was placed vertically in the midsagittal plane and participants saw a mirror reflection of their active hand. Direct vision of the inactive hand was not possible due to the positioning of the mirror; however, the mirror image of the active hand appeared superimposed on top of the obscured inactive hand. A custom-built stand, situated between participants' upper body and their active hand, also prevented direct vision of the active hand (Figure 5.1.). Auditory feedback in the form of a high or a low pitch tone was provided after each trial, informing participants whether peak acceleration on the preceding trial had been better (high tone) or worse (low tone) than the previous trial. Participants were familiarised with both tones before the start of the experiment to ensure their ability to distinguish them. The experimenter encouraged participants on

a regular basis and reminded them to “move as fast as possible” and to “produce / achieve as many high tones as possible”. A rest period of 30 seconds was given every 15 trials, therefore dividing the training period into 20 sub-blocks. We collected participants’ neurophysiological and behavioural data bilaterally after each training block (i.e., mid-test and post-test respectively) in a counterbalanced order (right/left hemisphere and right/left hand), but with the neurophysiological testing always preceding behavioural testing. Figure 5.2. outlines the experimental procedure.

Figure 5.1. Experimental setup for the visual feedback conditions

For the Mirror Vision condition (left) a mirror was placed vertically in the midsagittal plane and participants viewed a mirror reflection of their active hand, with the mirror image of the active hand appearing superimposed on top of the obscured position of the inactive hand. A custom-built screen, situated in the coronal plane between participants’ upper body and their active hand, further prevented a direct view of the active hand. For the Active Vision condition (right) the mirror was replaced with an opaque board.

5.3.4. Electromyographic recordings

Bilateral electromyographic (EMG) recordings were obtained from the left and right first dorsal interosseus (FDI) muscles, the primary muscle responsible for the finger abduction task. Participants' skin was prepared with a lightly abrasive gel and cleaned with an alcohol wipe before attaching Ag/AgCl electrodes (Meditrace 130, Tyco Healthcare, Mansfield, MA) in a belly-tendon montage. EMG signals were amplified (X1000) and a notch filter (50 Hz) was applied prior to sampling using a 16-bit AD system (Power 1401, CED Limited, Cambridge, UK). Collected data was stored on a computer for subsequent offline analysis.

5.3.5. Transcranial magnetic stimulation

TMS was used to investigate corticospinal excitability and short-interval intracortical inhibition (SICI) of the motor pathways from the left and right motor cortices (*l*M1 and *r*M1) at three different time points (i.e., before, between and after the two training blocks). TMS was delivered by two Magstim 200 magnetic stimulators (Magstim Company, UK) connected by a Bistim unit and a figure-of-eight coil (70 mm diameter). The optimal positions on the motor cortex (i.e., motor hotspots) at which a suprathreshold stimulation consistently elicited the largest motor evoked potentials (MEPs) in the left and the right FDI were determined and marked on the scalp. Resting motor thresholds (RMT), defined as the lowest TMS intensity needed to elicit at least three out of five MEPs ≥ 50 μV (Carroll, Barry, Riek, & Carson, 2001; Reissig et al., 2014; Rossini et al., 1994), were then determined for both target muscles using a posterior-to-anterior coil positioning (i.e., coil at ~45° to the midline and in a

plane tangential to the scalp surface leading to a posterior-to-anterior-induced current in the cortex).

During all three TMS sessions we administered 20 alternating single-pulses and paired-pulses to the motor cortices of the left and right hemisphere. Ten single-pulses were applied to assess corticospinal excitability using a suprathreshold stimulation intensity (130% RMT), and ten paired-pulses were applied to assess intracortical inhibitory processes. SICI was measured by applying a subthreshold conditioning stimulus before a suprathreshold test stimulus (130% RMT) with an interstimulus interval (ISI) of 3 ms (Kujirai et al., 1993). Following Garry and Thomson (2009) a fixed test (130% RMT) and fixed conditioning stimulus intensity (70% RMT) was employed to measure SICI. The ratio of the paired-pulse to single-pulse MEP amplitudes was used as an indication level of SICI.

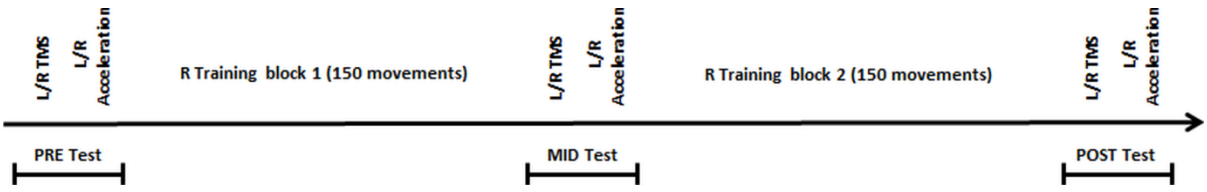

Figure 5.2. Experimental timeline: L = left R = right

5.3.6. Data acquisition and analysis

Custom-written CED (Cambridge, UK) Signal programs were used to sample kinematic and EMG data of each finger movement at 2 kHz for a duration of 1500 ms starting at 500 ms before the “go” tone. Acceleration data were low-pass filtered at 20 Hz prior to analysis, and peak acceleration was defined as the first peak in the horizontal acceleration.

Raw horizontal peak acceleration values were determined for both the left and the right hand at pre-, mid-, and post-test (*ACC*) and averaged across the ten trials. Peak acceleration obtained at mid- and post-test was normalised (*nACC*) to those values obtained at pre-test for each hand (i.e., trained hand acceleration was normalised to $ACC_{trained}$ in the pre-test, untrained hand acceleration was normalised to $ACC_{untrained}$ in the pre-test). A value of 1 was subsequently subtracted from these normalised accelerations to yield normalised change ($\Delta ACC_{untrained,}$ $\Delta ACC_{trained}$).

Peak acceleration, obtained during the two training blocks, was calculated in a similar way. For each of the 20 sub-blocks (see *experimental design*) we calculated an average raw peak acceleration value (*ACCtraining*) obtained during training. The average raw peak acceleration value (i.e., *ACCtraining* – see Methods) from the penultimate block (training period 2: movements 270 - 285) was then normalised to the average raw peak acceleration value from the first block (training period 1: movement 1 – 15) to obtain a variable (*nACCtraining*) describing performance gains in the trained hand over the duration of training using a single variable**.**

Responses to TMS at all three test points were excluded from further analysis if root-mean-squared EMG values exceeded 0.025 mV in the period 115 – 15 ms prior to each TMS pulse. In the remaining trials, the peak-to-peak MEP amplitudes elicited in the FDI contralateral to the stimulated hemisphere were calculated in the 50 ms window commencing 15 ms after TMS delivery. Single-pulse MEP amplitudes (*MEP*) were averaged and normalised to the MEPs obtained during the pre-test in each hand on a participant-by-participant basis (*nMEP*). Paired-pulse MEP amplitudes in both hands were determined for each trial in the pre-, mid-, and post-test and divided by the corresponding *MEP* of the same test-block to calculate a SICI ratio for each test-block (*SICI*). Accordingly, SICI < 1 indicates inhibition is present, with lower SICI

indicating greater inhibition. The same procedure as described above for MEPs was then applied to calculate normalised SICI (*nSICI*).

EMG data of the two training blocks were rectified and low-pass filtered (20 Hz) and subsequently analysed to quantify the movement-related muscle activity (trained hand) and corresponding mirror activity (untrained hand) prior to and during training. The peak EMG amplitude of the trained hand was determined and movement onset and offset were defined as the time at which EMG activity first increased above 4X the background EMG determined before movement onset and the time at which muscle activity of the active FDI first dropped below 0.2X the peak amplitude respectively (Carroll et al., 2008; Hinder et al., 2011). The average EMG activity of the trained (active) hand ($EMGtraining_{trained}$) was then calculated for this time-period, minus the background EMG exhibited prior to movement onset for each trial in the pre-test and in both training blocks respectively. The average EMG activity of the untrained (inactive) hand ($EMGtraining_{untrained}$) was established for the same time period using movement onset and offset as calculated above. For the training trials only, we then normalised the mirror activity in the untrained hand (as calculated above) to the EMG in the trained hand (for the same time period) for each trial. This method allowed us to refer to EMG activity in the inactive hand expressed as a percentage of the EMG activity in the active hand. We then averaged across all training trials to yield one value that represented the extent of mirror activation during training ($EMGtraining_{untrained}$).

5.3.7. Statistical Analysis

We separately analysed our test- and training-related dependent variables relating to task performance (*ACC, nACC, ΔACCtraining*), cortical excitability (*MEP, nMEP*) and inhibition SICI (*SICI, nSICI*), and volitional muscle activity during the motor task ($EMGtraining_{untrained}$) in multiple steps using various mixed model and between subject analyses of variance. Specifically, 2 (hand: left, right) x 2 (feedback: Mirror Vision, Active Vision) x 2 (age: younger, older) analyses of variances (Mixed ANOVAs) were initially employed in order to check for differences at pre-test for *ACC*, *MEP* and *SICI.* Next, in order to investigate test-related behavioural and neurophysiological changes in the trained and the untrained hand and hemisphere (relative to the pre-test), we subsequently examined *nACC*, *nMEP* and *nSICI* using 2 (time: mid, post) x 2 (hand: left, right) x 2 (feedback: Mirror Vision, Active Vision) x 2 (age: younger, older) analyses of variance (Mixed ANOVAs) for each dependent variable separately. In addition, we interpreted training-induced changes from pre-test to mid-test based on confidence interval assessment. In a next step we investigated changes in task performance in the trained hand as well as differences in the average level of EMG (mirror) activity in the untrained hand during training performing separate 2 (feedback: Mirror Vision / Active Vision) x 2 (age: younger, older) between-subject analyses of variance using *ΔACCtraining* and $EMGtraining_{untrained.}$ Separate multiple regression analyses for each age group were employed to identify main predictors of $\Delta ACC_{untrained}$, and to assess a possible relationship between $\Delta ACC_{untrained}$ and training-related variables *ΔACCtraining* and $EMGtraining_{untrained}$. Furthermore, we were also interested in possible relationships between $\Delta ACC_{untrained}$ and the test-related variables $\Delta ACC_{trained}$, $nMEP_{trained}$, $nMEP_{untrained}$, $nSICI_{trained}$, and $nSICI_{untrained}$ which represent changes in test

performance and neural excitability/inhibition that occurred as a function of training. The two training-related variables (*ΔACCtraining* and $EMGtraining_{untrained}$) were entered into the regression analysis (Enter Method) as a first cluster of potential predictors of $\Delta ACC_{untrained}$, and subsequently complemented by a second cluster of predictors using the test-related variables ($\Delta ACC_{trained}$, $nMEP_{trained}$, $nMEP_{untrained}$, $nSICI_{trained}$, and $nSICI_{untrained}$).

Data were checked for outliers (> 3 *SD*), which were removed prior to each analysis. Each variable was tested for normality using the Kolmogorov-Smirnov test, and log transformed (*ln*) in the event of a violation of normality prior to further analysis. The alpha level was set to 0.05 (with a Greenhouse-Geisser degrees of freedom adjustment applied when the assumption of sphericity was violated, i.e., $\varepsilon < 0.7$); significant main effects and interactions were explored using post-hoc pairwise comparisons using the Sidak adjustment. Partial eta-squared and Cohen's *d* are provided as measures of effect size to aid the interpretation of tests of significance. All data are presented as means and 95% confidence intervals (CI).

5.4. Results

5.4.1. Performance at pre-test and subsequent changes in performance with training

An initial analysis on *ACC* revealed a significant hand x age interaction, $F(1,49) = 12.21$, $p = 0.001$, $\eta_p^2 = 0.199$. Post-hoc comparisons revealed that while for the younger adults acceleration was greater in the trained hand (M = 0.34 [0.28, 0.41]) than in the untrained hand (M = 0.25 [0.17, 0.33]) ($p = 0.001$, $d = 1.273$), there was no such between-hand difference in the older adults ($p = 0.193$). Main effects of hand, feedback and age, and all other interactions were not statistically significant (all $F < 2.60$, all $p > 0.113$).

A subsequent analysis on *nACC* revealed a significant main effect of time, $F(1,49) = 29.27$, $p < 0.001$, $\eta_p^2 = 0.374$. Post-hoc comparisons showed that acceleration was greater at post-test (*M* = 1.46 [1.36, 1.57]) when compared to mid-test (*M* = 1.28 [1.20, 1.36]), $p < 0.001$. Furthermore, an interpretation of 95% CI's indicated that acceleration was greater at mid-test than at pre-test for both the trained hand (M = 1.33 [1.23, 1.43]) and the untrained hand (M = 1.23 [1.14, 1.32]). A significant main effect of hand revealed greater acceleration in the trained hand (*M* = 1.48 [1.36, 1.60]) compared to the untrained hand (*M* = 1.27 [1.17, 1.36]), $F(1,49) = 14.96$, $p < 0.001$, $\eta_p^2 = 0.234$. In addition, the time x hand interaction was also found to be significant, $F(1,49) = 13.33$, $p = 0.001$, $\eta_p^2 = 0.214$. Post-hoc comparisons revealed that acceleration at mid-test did not differ between the trained hand (*M* = 1.33 [1.23, 1.43]) and the untrained hand (*M* = 1.23 [1.14, 1.32]) ($p = 0.081$, $d = 0.285$), while at post-test it was significantly higher in the trained hand (*M* = 1.63 [1.48, 1.78]) than the untrained hand (*M* = 1.30 [1.19, 1.41]) ($p < 0.001$, $d = 0.705$). The main effects of age, $F(1,49) = 2.54$, $p = 0.117$, $\eta_p^2 = 0.049$, and feedback, $F(1,49) = 1.12$, $p = 0.295$,

η_p^2 = 0.022, were not significant. No other significant interactions were found (all F < 2.19, all p > 0.146). Figure 5.3. represents the performance changes in the trained and untrained hand.

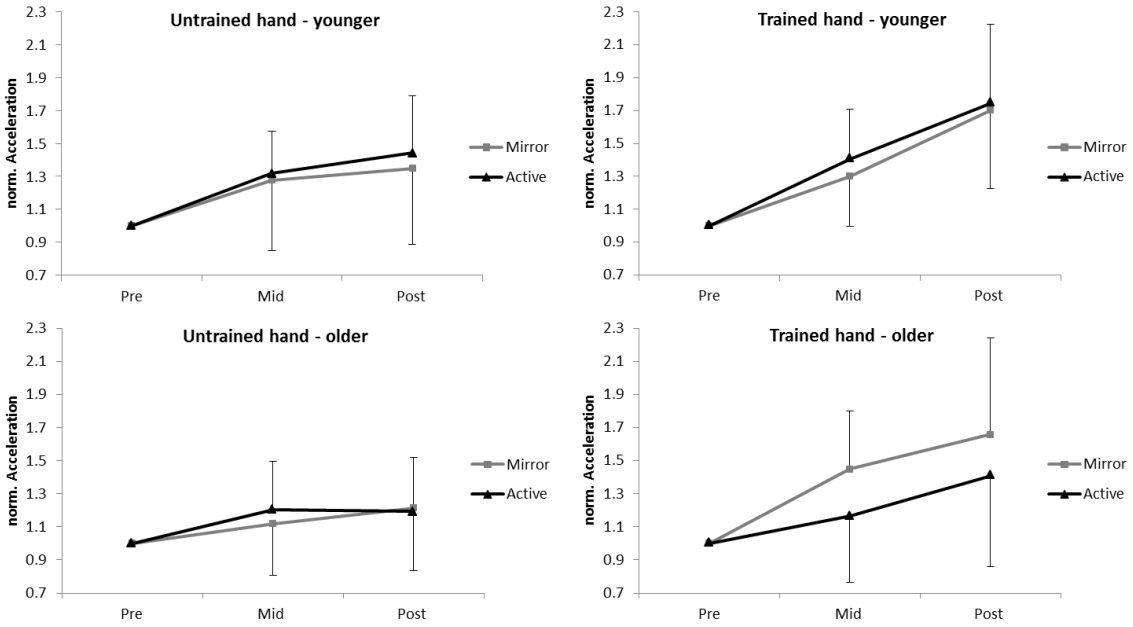

Figure 5.3. Performance changes trained and untrained hand (peak acceleration)

Normalised (*n*) performance of the untrained (left column) and the trained (right column) hand in the pre-, mid-, and post-test for the young (top row) and the older (bottom row) groups. Error bars denote SD.

5.4.2. Corticospinal excitability

An initial analysis on *MEP* revealed no significant main effects or interactions (all F < 1.74, all p > .194). Since the assumption of normality was violated (on the *nMEP* variable) log transformation was undertaken (i.e., *lnnMEP)* prior to further analysis. The analysis revealed a significant main effect of time, $F(1,44) = 4.11$, $p = 0.049$, η_p^2

= 0.084, with greater *lnnMEP* at post-test (*M* = 0.35 [0.24, 0.47]) compared to mid-test (*M* = 0.25 [0.14, 0.37]). In addition, an interpretation of 95% CI's indicated that *MEP* was greater at mid-test than at pre-test for both the trained hand (M = 0.32 [0.16, 0.48]) and the untrained hand (M = 0.19 [0.034, 0.35]). Analysis further revealed a trend for hand x feedback interaction, $F(1,44) = 4.01$, $p = 0.051$, $\eta_p^2 = 0.082$. Post-hoc comparisons revealed significantly higher *lnnMEP* in the hemisphere responsible for the trained hand (*M* = 0.49 [0.28, 0.69]) compared to the hemisphere responsible for the untrained hand (*M* = 0.13 [-0.09, 0.35]) in the AV condition, *p* = 0.025, *d* = 0.192. No other significant main effects or interactions were found (all *F* < 1.70, all *p* > 0.199). Figure 5.4. represents the changes in MEP amplitudes in both hemispheres.

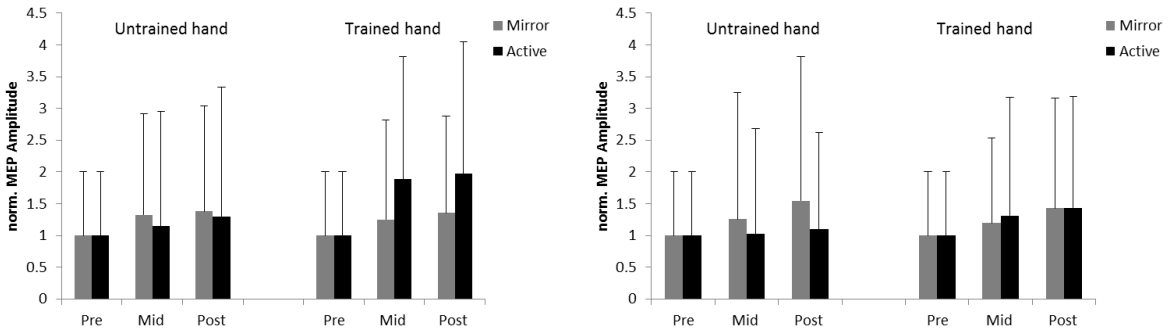

Figure 5.4. Changes in MEP amplitudes in both hemispheres

Normalised and back transformed (*nMEP*) amplitudes evoked in the FDI of the trained and the untrained hand for the younger (left side) and the older (right side) groups at pre-, mid-, and post-test. Error bars denote SD.

5.4.3. Short-interval intracortical inhibition at pre-test and subsequent changes

An initial analysis on *SICI* revealed a significant feedback x age x hand interaction, $F(1,44) = 4.49$, $p = 0.041$, $\eta_p^2 = 0.090$. Post-hoc comparisons revealed significantly lower *SICI* ratio (i.e., greater inhibition) in the trained hemisphere (M = 0.56 [0.14, 0.97]) compared to the untrained hemisphere (M = 1.13 [0.53, 0.1.73]) in the MV condition in the older adults, $p = 0.048$, $d = 0.0.7588$. No other significant main effect or interactions were found (all $F < 1.11$, all $p > 0.299$).

Subsequent analysis was performed on *lnnSICI* as the assumption of normality on *nSICI* was violated. Data analysis revealed no significant main effects or interactions (all $F < 2.16$, all $p > 0.149$).

5.4.4. Changes in performance in the trained hand during training

An analysis performed on *ΔACCtraining* revealed a significant feedback x age interaction, $F(1,49) = 8.11$, $p = 0.006$, $\eta_p^2 = 0.142$. Post-hoc comparisons indicated that in the AV condition significantly smaller performance increases were observed in the older participants ($M = 1.313$ [0.896, 1.730]) than in the younger group ($M = 2.150$ [1.700, 2.600]) ($p = 0.008$, $d = 1.473$). The behavioural change for the AV_{older} was also less pronounced when compared to MV_{older} ($M = 2.068$ [1.618, 2.518]) ($p = 0.017$, $d = 0.857$).

5.4.5. Mirror activation in the untrained hand during training

Based on our exclusion criteria (see *Analysis section*), one older participant from the MV group was excluded prior to analysis. Subsequent analysis was performed on $lnEMGtraining_{untrained}$ as the assumption of normality was violated. The level of mirror

activity did not differ significantly between the younger and older participants or as a consequence of the provided feedback during training (all $F < 1.19$, all $p > 0.282$).

5.4.6. Predictors of performance change in the untrained hand

Separate multiple regression analyses were employed to identify significant predictors of $\Delta ACC_{untrained}$ for the younger and the older adults. For the older adults, analysis revealed that $\Delta ACC_{untrained}$ was significantly predicted by both models (i.e., with and without inclusion of the test-related variables – see Methods). The model excluding the test-related variables revealed a better fit and significance (adjusted $R^2 = 0.51$, $F(2,19) = 11.81$, $p < 0.001$) than the model including all (i.e., test and training) variables (adjusted $R^2 = 0.50$, $F(6,15) = 4.47$, $p = 0.009$). In the younger adults $\Delta ACC_{untrained}$ was significantly predicted by the model that included the training-related variables (adjusted $R^2 = 0.17$, $F(2,22) = 3.51$, $p = 0.047$), but not by the model that was complemented by the test-related variables ($\Delta R^2 = 0.19$, $\Delta F(4,18) = 1.54$, $\Delta p = 0.233$). In the older adults, $lnEMGtraining_{untrained,}$ $\beta = 0.604$, $t(2,19) = 3.83$, $p = 0.001$, uniquely accounted for a significant portion of the variance in $\Delta ACC_{untrained}$, explaining 36.5% of the variance. In addition, *ΔACCtraining* was marginally associated with changes in $\Delta ACC_{untrained}$, $\beta = 0.315$, $t(2,19) = 2.00$, $p = 0.061$, explaining a further 9.9% of the variance. In the younger adults, only *ΔACCtraining* accounted for a significant portion of the variance in $\Delta ACC_{untrained}$, $\beta = 0.496$, $t(2,22) = 2.63$, $p = 0.015$, explaining 24.6% of the variance (Figure 5.5.).

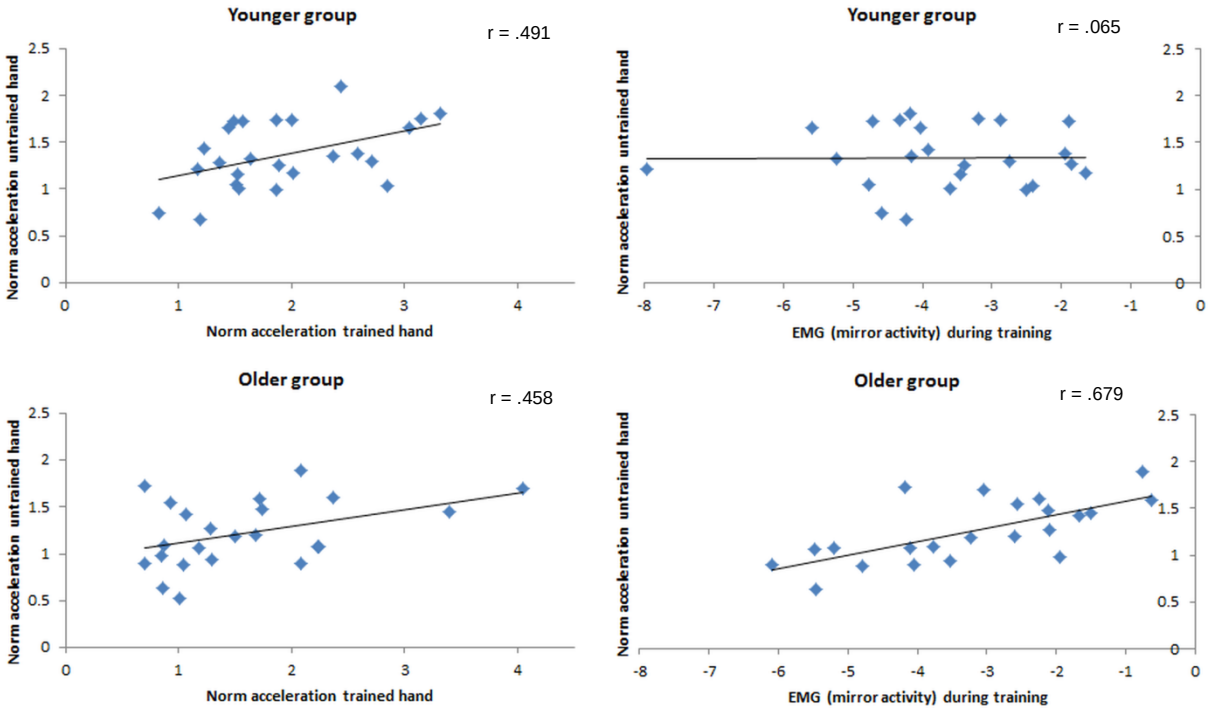

Figure 5.5. Correlation between behavioural and neurophysiological parameters

Simple Correlation (i.e., zero-order Correlation) between the change of performance in the untrained hand at post-test ($\Delta ACC_{untrained}$) and a) the change of performance in the trained hand during training (*ΔACCtraining*) (left side) and b) the average amount of EMG activity exhibited in the untrained hand ($lnEMGtraining_{untrained}$) during training (right side) for the younger group (top row) and the older group (bottom row).

5.5. Discussion

This study engaged younger and older adults in a ballistic motor training paradigm with their dominant hand and investigated subsequent performance gains (in the same task) in the dominant and the non-dominant hand (i.e., CLT). During the motor learning period participants were provided with different types of visual feedback, either focussing on their active hand, or focussing on a mirror reflection of their active hand, with the aim of facilitating CLT effects.

Participants in both age groups demonstrated an increase in task performance (i.e., peak acceleration) over the duration of the experiment which, in line with previous work (Dickins et al., 2015; Hinder et al., 2011; Hinder et al., 2013; Dickins et al., 2015), was accompanied by a bilateral increase in corticospinal excitability. Further, and consistent with previous findings (for an overview see Carroll et al., 2006; Lee et al., 2010), the increase in task performance was found to be greater in the trained hand (63% improvement) than in the untrained hand (30% improvement). Moreover, older adults in the current study displayed CLT to an extent that was comparable to the young adults. The current findings therefore suggest that older adults, contrary to previous results (Hinder et al., 2011; Parikh & Cole, 2013), are capable of showing cross-limb-transfer-effects to a similar degree to those exhibited by younger adults, a result supported by a recent study by Dickins et al. (2015) that has also demonstrated preserved transfer for older adults in both a complex and a simple motor task.

Unlike the current experiment, where we specifically asked participants to either continuously focus on their active hand or on a mirror image of their active hand during task performance, neither of the two previous studies (Hinder et al., 2011,

Parikh & Cole, 2013) provided explicit instructions with regard to the focus of attention during the training. However, the provision of feedback about task performance on a computer screen in those studies suggests that at least some focus was directed away from the hands. As older adults have been shown to be more reliant on visual feedback for accurate motor performance (Swinnen et al., 1998; Voelcker-Rehage, 2008), it is possible that for our older adults focussing on the active hand or a mirror image of the active hand (rather than focussing on a computer screen) represented a beneficial factor leading to similar performance gains in the trained and untrained hand as the younger adults. Indeed, in younger participants, prior observation of a motor action has been shown to be beneficial for subsequent motor learning in the absence of movement execution (Mattar & Gribble, 2005; Stefan et al., 2005). With regard to ageing, Celnik et al. (2006) previously demonstrated that combining action observation and motor training augmented those training effects obtained by motor training alone, conceivably through a strengthened input to M1 from ventral premotor cortex (through action observation) and supplementary motor area and dorsal premotor cortex (through action execution). It is conceivable, therefore that for the older adults in previous studies (Hinder et al., 2011; Parikh & Cole, 2013), not focusing continually on the hands – while not appearing to decrease performance gains in the *trained* hand - affected the mechanisms of learning and, thus, affected (precluded) subsequent transfer. We acknowledge the possibility that our group sizes, although common in TMS studies (e.g., Dickins et al. 2015; Hinder et al., 2011, 2013; Parikh and Cole, 2013), may have contributed to the absence of statistically significant effects of age. Nonetheless, we believe that a continuous focus on the executing hand (as applied in the current study) might have facilitated our participants to internalise crucial

movement parameters more effectively, subsequently enabling them to also show performance improvements in the *untrained* hand.

For the current task the provision of augmented visual feedback via MVF, rather than 'standard' visual feedback, did not significantly enhance CLT in our younger or older participants. It is possible that our ballistic finger movement task did not elicit enhanced performance improvements in the untrained hand in the MVF condition, because online visual feedback was neither a prerequisite for accurate completion of the task, nor was it necessary to drive performance improvements. It is conceivable that the provision of visual feedback was helpful during the very early stages of the training period, in which participants acquired the basic 'structure' of the simple movement task, but the feedback did not contribute to subsequent performance improvements. MVF may promote enhanced learning and facilitate CLT if used in conjunction with a more demanding task (perhaps requiring online modifications and feedback control) in which visual feedback has been shown to be most beneficial (for an overview see Sigrist, Rauter, Riener, & Wolf, 2013). Consistent with this supposition, in recent studies showing beneficial effects of mirror-visual feedback on CLT, participants were engaged in motor tasks requiring and / or profiting from online visual feedback, such as moving marbles with a spoon, putting elastic bands over a glass, or rotating two balls in one hand as quickly as possible (Lappchen et al., 2012; Nojima et al., 2012). Transfer in tasks involving forceful contractions, such as the ballistic task employed here, or strength training protocols (e.g., Farthing et al., 2007) may be less influenced by manipulations in visual feedback.

While the lack of a significant effect of visual feedback in mediating the extent of CLT may have been caused by a lack of task complexity, it is also possible that other

factors may be more important in facilitating CLT across the lifespan. In the older adults the extent of bilateral activation (mirror activity) during the training period significantly accounted for subsequent performance gains in the untrained hand (accounted for 36.5% of the variance), while the extent of performance improvements in the trained hand was weakly associated with the subsequent transfer. The current results thus suggest that, for older adults, unintended activation of the ipsilateral hand during unilateral training appears to be crucial in increasing subsequent motor performance in the untrained hand. Greater bilateral muscle activity during unilateral tasks in the older adults is well known and has previously been shown for a variety of different movement tasks (Baliz et al., 2005; Bodwell et al, 2003; Hinder et a., 2011; Hoy et al., 2004; Mattay et al., 2002; Ward & Frackowiak, 2003). This increase in motor overflow is most likely caused by neurological changes in the healthy ageing brain, such as a reduced integrity of the corpus callosum (Hoy et al., 2004) resulting in bilateral cortical activity. Overactivation of (bilateral) brain areas not primarily involved for task execution has previously been shown to be associated with better task performance in the elderly in studies employing simple (Mattay et al., 2002; Ward & Frackowiak, 2003) and complex motor tasks (Bodwell 2003). In line with those experiments, the current study suggests that bilateral muscle activation is also important for the *transfer* of simple motor tasks in older adults (Figure 5.5.). In accordance with the HAROLD model (Cabeza, 2002), which describes less lateralised prefrontal activation to be associated with increased cognitive task performance in older adults, we propose that the exhibited bilateral activation can be considered a compensatory mechanism to ensure bilateral performance improvements after unilateral movement tasks. Despite the fact that the task under investigation (suggested to be predominantly

M1-driven) did not result in age-dependent changes in M1, according to the above mentioned studies we assume that an unconscious bilateral cortical activation in other brain areas (not assessed here) that project directly or indirectly onto M1 may have promoted bilateral muscle activity in the older adults and ensured performance improvements in both the trained *and* the untrained hand. Support for this hypothesis comes from previous studies that (additionally to prefrontal areas) have shown greater bilateral activation in sensorimotor cortex during unilateral motor tasks to be beneficial for motor performance in older adults (Mattay et al, 2002; Naccarato et al., 2006). Our proposal of bilateral activation being a compensatory rather than a mal-adaptive mechanism for CLT in the older adults is also in accordance with a study by Graziadio et al. (2015). In their study the authors found *similar* transfer in young and older groups in movement tasks that have been shown to result in bilateral cortical activation in both age groups, but *increased* transfer in the older compared to the younger adults for movement tasks that are known to cause unilateral activation in the younger and bilateral activation in the older adults. Based on those results it was suggested that the age-related bilateral activation involved the recruitment of neural circuits available to both hands and therefore facilitated subsequence transfer of the learned motor skill from the trained to the untrained hand. The current findings support and extend those obtained by Graziado et al. (2015) through neurophysiological results.

In the current study, whereas older adults' performance gains in the untrained hand seem dependent, at least to some degree, on prior unintended muscle activation of that hand, CLT does not appear contingent upon bilateral activation in the younger adults. This latter result is in accord with a recent review that has suggested bilateral activation as a non-essential process for CLT in young people (Zult, Howatson,

Kadar, Farthing, & Hortobagyi, 2014). Rather, and consistent with previous research (Hinder et al., 2012; Hinder et al., 2011; Lee et al., 2010), in the current study younger adults' performance increases in the untrained hand were found to be contingent upon (i.e., correlated to) training related gains in the trained hand (accounted for 24.6% of the variance). That is, younger participants showing the greatest performance gains during training subsequently demonstrated higher increases in motor performance in the untrained hand.

In light of the current results, changes in the nature of the neural mechanisms mediating CLT may occur as part of the healthy ageing process. With regard to potential mechanisms of CLT, current hypotheses either suggest changes in the untrained hemisphere (i.e., cross-activation hypothesis) or changes in the trained hemisphere, accessible by the untrained hemisphere (i.e., bilateral access hypothesis) as a requirement for successful transfer (for more detail see Lee et al., 2010). Our results demonstrate bilateral increases in corticospinal excitability following unilateral practice across all groups; however, these increases did not predict the extent of CLT. This finding is in accordance with a previous study by Dickins et al. (2015), who also only found a marginally-reliable relationship between changes in corticospinal excitability in the untrained hemisphere and CLT in the younger and no such association in the older adults. Rather, a strong relationship between mirror activation and performance changes in the untrained hand in the older participants was observed. Accordingly, it may be the case that bilateral muscle activity recorded *during* a task is a more sensitive marker of bilateral activation than measures of corticospinal excitability determined at rest following training, which may be influenced by numerous other factors. While increases in corticospinal excitability in the untrained hemisphere were causally related to transfer

in younger adults in a previous study (Lee et al., 2010), the fact we did not find a similar relationship indicates that the relationship between performance increases and excitability may be quite variable.

The finding of a significant relationship between mirror activity and CLT is consistent with the cross-activation theory (Carroll et al., 2008; Hinder et al., 2011; Lee et al., 2010), whereby unilateral-training-induced activations in the trained and untrained hemispheres specifically mediate the contralateral (i.e., trained or untrained) limb's performance, respectively (Lee et al., 2010). That is, activation of the untrained hemisphere is likely to drive successful CLT and subsequent performance improvements in the untrained hand (Carroll et al., 2008; Hinder et al., 2011; Lee et al., 2010; Wiestler, Waters-Metenier, & Diedrichsen, 2014). Interestingly, our findings demonstrating an association between bilateral muscle activation and cross-limb transfer in the older adults appear contradictory to the results obtained by an earlier study (Hinder et al., 2011) at first view, which did not find any such relationship. However, while bilateral EMG activity in the current study was recorded during the training period, in the experiment conducted by Hinder et al. (2011), it was collected during the test-period. The possibility therefore exists that, in line with the cross-activation hypothesis, processes (i.e., bilateral muscle activation) occurring *during* the actual intervention period, but not *after* training, might drive cross-limb adaptations and thus underpin subsequent cross-limb transfer. In the current study, despite the fact that young and older adults exhibited comparable levels of performance increase in the trained hand and similar levels of mirror activity in the untrained (inactive) limb, the finding that these parameters relate differently to subsequent CLT is suggestive of subtle changes in the factors mediating CLT. Moreover, possible changes in the balance of the mechanisms underlying transfer,

together with shifts in the level of action of these mechanisms (e.g., cortical, corticospinal or spinal) may occur as a function of healthy ageing.

Considering the purported changes in the mechanisms underlying CLT that occur across the lifespan, future research is warranted comparing the neuronal activation of regions, such as M1 or dorsal premotor cortex, which are presumably activated during the learning and retrieval of a unilateral movement task and the subsequent performance in the untrained hand in younger and older adults. Based on the current finding that bilateral (muscle) activation appears to be a driving factor in eliciting CLT in the older (but not the younger) adults, future work could also aim to manipulate the amount of mirror movements during a unilateral ballistic movement task and assess the corresponding change in transfer. Non-invasive brain stimulation techniques could be used to up- or down-regulate presumably active brain regions in order to elucidate causal relationships between CLT and mirror activation. Such findings would improve the understanding of the neural underpinnings of CLT and subsequently enhance clinical applicability. Finally, to further investigate the effects of augmented visual feedback (i.e., MVF) on CLT, future work could focus on a variety of more complex tasks requiring online modification of motor commands on the basis of visual feedback. Having demonstrated that focusing attention on either the active hand or a mirror image of the active hand *can* evoke transfer in older adults, such an approach would enable us to determine whether further enhancement of transfer by way of MVF is possible for certain tasks that mimic complex everyday movements (e.g., reaching and grasping), which are vital to maintain independent living in later life, but often affected severely following brain injury (e.g. stroke) or following falls and subsequent limb immobilisation.

CHAPTER 6: GENERAL DISCUSSION AND CONCLUSION

The aim of the experimental research reported within this thesis was to investigate the role of visual feedback in modulating CSE and performance gains, in the trained and untrained hemisphere and hand following unilateral motor training, in younger and older adults. More specifically, I was interested in further elucidating the effects of MVF on neurophysiological and behavioural parameters in comparison to more usual forms of visual feedback, such as focussing on the hand executing the (unimanual) motor task. To this end, participants were asked to practice various unilateral motor tasks and were provided with different visual feedback during task execution. Subsequently, neurophysiological changes in circuits projecting from the trained and untrained motor cortex were investigated (via TMS measurements) (chapter 3 and chapter 5, as well as follow-up studies 1-3), together with behavioural changes in both limbs (via performance tests) (chapter 4 and chapter 5). In addition, I was interested in the degree to which ageing and associated changes in brain structure and function would alter the effect of such MVF-induced changes in both behavioural and neurophysiological factors (chapter 3 and chapter 5). Investigating these topics is relevant for the use of MT as a potential clinical tool in general (e.g., stroke or rehabilitation from unilateral injuries such as limb fracture), and to specifically treat age-related disorders.

6.1. Summary of results

Chapter 3 reported a study investigating the degree to which MVF of a moving limb was capable of eliciting a greater enhancement of movement-related CSE changes in the ipsilateral (inactive) hemisphere, compared to more standard visual feedback, in younger and older adults (N = 24). It was found that MVF, irrespective of age, did not lead to more pronounced changes in ipsilateral CSE or intracortical inhibition compared to when visual focus was put on the active or passive limb (all p's > 0.911). These findings are contrary to an earlier study by Garry et al. (2005), but are in agreement with other more recent studies (Avanzino et al., 2014; Carson & Ruddy, 2012; Funase et al., 2007) that also failed to find a superior effect of MVF on ipsilateral CSE. Based on the results described in chapter 3 (and published as Reissig et al., 2014*, Neuropsychological Rehabilitation*), it was concluded that enhanced CSE in the hemisphere ipsilateral to a moving limb is unlikely to represent the primary mechanism underlying behavioural improvements in the untrained limb which have been shown to be facilitated by MVF.

Chapter 4 aimed to confirm previous findings of MVF-induced facilitation of CLT in younger adults (N = 80) with respect to learning of a complex motor task. Furthermore this study investigated whether such enhanced performance gains in the untrained limb are predominantly underpinned by mechanisms of action observation or more traditional motor transfer hypotheses. Unlike findings reported by two recent studies published during the period this thesis was undertaken (Nojima et al., 2012; Nojima et al., 2013) the present results failed to demonstrate MVF-related benefits in the degree to which CLT was exhibited following a complex unilateral ball rotation task (p = 0.520). However, the findings (published as Reissig

et al., 2015, *PLOS ONE*) do suggest that behavioural changes in the untrained hand following a unilateral training task with MVF are most likely induced due to a combination of crossed-effects (at the level of the motor cortex) mediating transfer of skill and action observation effects related specifically to the nature of the feedback.

Chapter 5 addressed CLT in a simple ballistic motor learning task in young and older populations. In contrast to the complex task described in chapter 4, this ballistic task has been shown to share neural attributes with strength training (Selvanayagam et al., 2011), and is thus an appropriate model to investigate an important motor attribute which may be lost in older age and following traumatic injury. Despite the fact that this experiment suggested that behavioural and neurophysiological effects did not vary specifically with the provision of MVF (all *p's* > 0.1) the study importantly showed that, unlike previously reported (Bemben & Murphy, 2001; Hinder et al., 2011), older adults are able to exhibit a similar degree of CLT to younger adults. Interestingly, despite similar CLT values, correlation analyses revealed different movement parameters predicted CLT for each age group. While CLT in the younger adults was found to be predominantly associated with performance gains in the trained hand (β = 0.47), the amount of mirror activity exhibited during learning was related to subsequent CLT in the older adults (β = 0.60) (Reissig et al., under review at *Frontiers in Aging Neuroscience*). Consistent with other very recent studies (Dickins, Sale, & Kamke, 2015; Graziadio et al., 2015), the results of this experiment demonstrated a preservation of the behavioural consequences of CLT in the untrained limb in older adults, albeit possibly via a different neural mechanism to that exploited in younger adults. Moreover, the results suggest that bilateral activation, often reported within the context of unilateral motor tasks in the ageing population, may be compensatory with respect to permitting CLT behavioural gains.

6.2. Implications of results for mirror-visual feedback research

6.2.1. The influence of mirror-visual feedback on corticospinal excitability

Summarising the results of the four studies (i.e., study 1 and follow-up studies 1-3 – presented in chapter 3), which assessed changes in cortical mechanisms during a simple repetitive motor action, it appears that visual feedback can alter CSE to some extent. Specifically, provision of vision of the active hand or its mirror image (and asking the participant to maintain visual attention on that feedback) elicited greater CSE change compared to fixation on a central cross. This finding is in agreement with previous studies that reported changing the focus of attention led to changes in cortical plasticity (Kamke et al., 2012; Sale & Mattingley, 2013). However, the results of the current project suggest that MVF does not lead to ipsilateral CSE increases that outweigh those observed when other types of visual feedback are provided. These results suggest that enhanced CSE or activation in the motor cortex ipsilateral to a moving limb, are unlikely to represent a critical mechanism underlying MVF-related behavioural changes. This conclusion contrasts with the original work from Garry et al. (2005), but is supported by other TMS and fMRI research (Avanzino et al., 2014; Fritzsch et al., 2014; Funase et al., 2007; Mehnert, Brunetti, Steinbrink, Niedeggen, & Dohle, 2013; Wang et al., 2013; Zult, Goodall, Thomas, Hortobagyi, & Howatson, 2015).

In addition to the absence of MVF-related changes in CSE, the studies also failed to demonstrate MVF-specific effects in either intra- or intercortical inhibition (SICI and IHI, respectively). Despite the fact that the current SICI results are in agreement with other studies (Carson & Ruddy, 2012; Lappchen et al., 2012; Nojima et al., 2012), it should be mentioned that a recent study by Zult et al. (2015) reported more

pronounced decreases in SICI when provided with MVF compared to when vision of either hand (i.e., passive and active) was occluded. Despite being significant, the additional decrease in SICI in their study was rather small (i.e., 9%). Moreover, their participants were required to produce substantially higher forces (i.e., approximately 60% maximal voluntary contraction) than in the four present studies. Accordingly, Zult et al. findings suggesting a MVF-induced modification of SICI might be specific to their task and not be able to be generalised to more repetitive or low force tasks. The results of follow-up study 3 seem to suggest that changes in IHI may also not represent a primary mechanism underlying MVF-induced behavioural changes. However, the recent report of MVF-induced IHI changes in a similar task to that employed in the initial studies of the present study (Avanzino et al., 2014) suggests the possibility that IHI changes may have occurred in the other direction (i.e., from inactive to active hemisphere rather than active to inactive as was investigated in follow-up study 3). Accordingly the role of IHI in the experiment remains inconclusive.

Despite the fact that we only tested a subset of possible TMS protocols (e.g., no LICI, or SICF measurement) it is conceivable that changes in net excitability (as elucidated by traditional TMS measurements) might not fully represent the beneficial effect of MVF, due to changes occurring upstream of M1 in secondary and preparatory motor regions, or within the parietal or occipital lobe. This idea is in accordance with previous imaging studies (Dohle et al., 2004; Fink et al., 1999; Fritzsch et al., 2014; Hamzei et al., 2012; Wang et al., 2013). That is, the output of M1 may be maintained due to MVF-specific changes that occur upstream of M1.

Alternatively, it is also conceivable that no significant MVF-induced changes in CSE were observed in the present work due to a weak, or absent, association between CSE increases in the ipsilateral hemisphere and subsequently induced behavioural gains in the corresponding inactive hand (in general and with regards to MVF specifically). Indeed, while this association is often assumed, to the best of my knowledge only one study has provided direct evidence of a correlation between MVF-induced CSE changes and subsequent performance gains in the untrained hemisphere and side, respectively (Nojima et al., 2012). While this result is a step towards understanding the role of ipsilateral CSE change, it should also be noted that the study was only correlational, and did not provide evidence of a *causal* association between ipsilateral CSE changes and behaviour. CSE increases in the inactive hemisphere, as measured via TMS, may indeed only partially contribute to subsequent performance gains in the corresponding limb, and CSE measurements via TMS from M1 might thus not be an ideal parameter to investigate the underlying neural mechanism responsible for MVF-induced behavioural changes.

Finally, it is conceivable that specific MVF-induced changes in CSE, SICI and IHI were not found in any of the four studies due to the task being of a repetitive, non-goal orientated nature. For the experiments reported in chapters 4 and chapter 5, it was decided to change the type of intervention, and employ a more performance driven task required learning to occur. It was thought that such a task would clarify whether the lack of MVF-specific changes in the investigated neurophysiological mechanisms was indeed due to the use of tasks that were not goal-orientated. Furthermore, employing a task that required learning also allowed for additional behavioural measurements to be collected and potential correlations between MVF-specific neurophysiological and behavioural changes to be investigated.

6.2.2. The influence of mirror-visual feedback on cross-limb transfer

Chapters 4 and 5 reported studies investigating the influence of MVF within both a complex (ball rotation) and a simple (finger acceleration) unilateral motor training paradigm, respectively. The focus was on the extent to which motor training affected performance in the untrained hand (by way of CLT) and to assess the associated neurophysiological changes in the ipsilateral (untrained) hemisphere. These studies built on chapter 3 which, rather than attempting to induce motor learning in the trained and untrained limb following unilateral motor training, simply assessed potential neurophysiological changes in the passive (ipsilateral) hemisphere during repetitive unilateral motor actions. In contrast to other recent reports (Lappchen et al., 2012; Nojima et al., 2012; Nojima et al., 2013), neither of the studies presented within this thesis found unique MVF-induced changes in the extent of CLT (performance) in the untrained limb, nor MVF-specific neurophysiological changes in the ipsilateral hemisphere. The absence of augmented CLT reported in chapter 5 might potentially be explained by the lack of complexity of the employed movement. Whereas complex motor tasks call for a certain amount of online modification and feedback control, simpler motor tasks, like the ballistic movement reported in chapter 5, most likely do not require online visual feedback (either standard or augmented) to complete the task accurately and/or drive subsequent performance gains. Consistent with this assumption, all the studies that previously found augmented feedback (i.e., MVF) to facilitate CLT engaged participants in more demanding complex tasks, such as moving marbles with a spoon, or rotating two balls in one hand as quickly as possible (Hamzei et al., 2012; Lappchen et al., 2012; Nojima et al., 2012). However, and despite employing the same complex motor task used previously (Nojima et al., 2012; Nojima et al., 2013), surprisingly, no increased MVF-induced CLT was found

in the ball rotation study reported in chapter 4. Thus, the complexity of the motor task is unlikely to represent the only factor determining whether MVF will lead to enhanced behavioural effects in the untrained limb.

Even though the studies reported in the present thesis were conducted with greater sample sizes than the majority of other studies in this research area, the results with regards to the specific behavioural and neurophysiological effects of MVF were variable and non-significant. Based on the findings it thus seems that the previously reported beneficial effects of MVF (Howatson et al., 2013; Zult et al., 2014) might not be as consistent or robust across tasks or participant cohorts as one would desire, especially if such protocols are, ultimately, to be adopted for rehabilitative programmes to assist in movement rehabilitation. Indeed, it is conceivable that the current impression that MVF consistently and robustly impacts positively upon CLT is influenced somewhat by publication biases towards reporting of positive results; something quite common in the scientific literature. This 'file drawer problem' (Rosenthal, 1979) has been understood for some decades, but has more recently again being discussed as a problem in a number of scientific and psychological fields (Deconinck et al., 2015; Earp & Trafimow, 2015; Rosenthal, 1979). Consequently, the effect of MVF on CLT might be dependent on a variety of different (concurrently occurring) factors, such as motivation, task challenge, differences in the level of engagement in the observation, as well as the degree of neurological impairment. Supportive of that idea, Fritzsch et al. (2014) also recently proposed that the training effect of MVF might not be different from more conventional movement therapies and that M1 plasticity induced by MVF might rather be considered a general training effect than an immediate, direct response to the mirror illusion.

Regardless of the apparent disagreement relating to the robustness and generalisability of MVF effects on CLT across different tasks and participant cohorts, the results reported in chapter 4 suggest that MVF-induced behavioural changes in the untrained limb most likely occur due to a combination of mechanisms related to the transfer of motor skills (crossed-effects at the level of the motor cortex) and action observation effects. It is conceivable that the degree of cross-activation (i.e., bilateral cortical activation resulting from unilateral motor activation) can be augmented due to the observation of the moving limb's mirror image. Such strengthened activation of the ipsilateral M1 and corticospinal tract might then in some cases lead to an enhanced degree of "traditional" CLT (for further information see Howatson et al., 2013). However, MVF-induced performance increases *without* unilateral training and associated gains in the trained hand are, according to the results of studies 3 and 5, not very likely. That is, performance increases in the untrained hand do not solely appear to be due to action observation in the absence of motor learning. Rather, as shown in both training protocols (i.e., simple and complex motor tasks) for the younger adults, performance gains in the untrained hand were associated with those training gains observed in the trained hand. This finding stresses the importance of the unilateral training more than the type of visual feedback provided during the intervention when aiming to induce bilateral performance improvements.

Attempts to measure the aforementioned additional MVF-related cortical excitation were unsuccessful in the studies reported in chapters 3 and 5. Despite corticospinal increases in the hemisphere ipsilateral to the moving limb, no additional increases were found that were specific to MVF either during or following to the performance of a simple motor task. One possible scenario explaining the lack of neurophysiological

changes related to the provided visual feedback might be the absence of MVF-specific behavioural improvements. However, as mentioned in the previous chapter, it is also possible that a relationship between changes in CSE and performance improvements in the corresponding limb in general and with regards to MVF specifically, unlike often assumed, might not actually exist. A recent review by Ruddy and Carson (2013), investigating the neural pathways mediating CLT, also highlighted this missing association between neurophysiological parameters (measured as changes in CSE using TMS) and behavioural factors (measured as performance gains in the untrained limb). The authors proposed two different, not mutually exclusive, scenarios in an attempt to explain such lack of apparent association. According to the first scenario, CSE increases might just simply be a reflection of crossed facilitation (i.e., bilateral cortical activation resulting from unilateral motor activation), whereas CLT (i.e., the behavioural change) occurs due to mechanisms upstream of M1. Alternatively, CLT could be mediated by interneuronal networks within the M1, which are not engaged in corticospinal output, and therefore not measurable via TMS. Based on either of the two scenarios as well as on the results reported in chapter 5 it seems that TMS measurements might not be an ideal tool to investigate CLT-induced neurophysiological changes in general and with regards to MVF specifically.

In summary, the results show that CLT as a consequence of unilateral training programmes cannot consistently be enhanced via augmented visual feedback (i.e., MVF). Although such training protocols might have the potential to be beneficial, such effects are influenced by many different factors that need to be controlled for and studied first in order to be able to predict the outcome of MT and employ it across a wider range of neurological disorders.

6.2.3. Cross limb transfer in the ageing population and possible MVF effects

Chapters 3 and 5 reported studies investigating the degree to which ageing might be associated with an altered influence of MVF in mediating changes on both neurophysiological and behavioural levels. Similar to the results obtained for the younger participants, no MVF-specific effects were found with regards to CSE changes or with regards to the subsequently exhibited CLT. Moreover, older adults did not show a different (i.e., greater) response with regards to either parameter compared to the younger adults. The potential reasons for the absence of beneficial MVF effects have been outlined above and are likely to apply just as much to older as to younger adults. The following sections will thus summarise only those findings that specifically relate to the context of ageing.

Even though MVF did not exhibit the capacity to augment CLT in the older adults when compared to more standard visual feedback conditions, the degree of transfer exhibited by the older adults in the simple motor task was found to be similar to that exhibited by younger adults. In light of previous findings that suggest ageing may be associated with decreased, or absence of, transfer of motor skills in a similar motor task (Hinder et al., 2011; Parikh & Cole, 2013), the current results are both interesting and important. Specifically, contrary to the hypothesis suggested in the introduction of the current thesis, the results suggest that task complexity itself *cannot* be the main factor predicting CLT in older adults. This conclusion is supported by two recent studies (Dickins et al., 2015; Goodwill, Daly, & Kidgell, 2015). Whereas Goodwill et al. (2015) failed to show CLT in a complex motor task in older adults, Dickins et al. (2015) demonstrated preserved transfer for older adults in both a complex and a simple motor task. In light of this inconsistency, it appears as

though factors other than task complexity have an influence on the degree of CLT exhibited by older adults.

Based on the results presented in chapter 5, one such factor that may influence the extent of CLT in older adults could be the amount of bilateral activation that is exhibited during the unilateral intervention. In accordance with previous studies (Bodwell et al., 2003; Mattay et al., 2002; Ward & Frackowiak, 2003) and the HAROLD model (Cabeza, 2002), it appears that the overactivation of (bilateral) brain areas might not only be associated with better task performance in older adults in general. Rather, it seems that bilateral activation might also be a compensatory mechanism to ensure bilateral performance improvements after unilateral movement tasks; a finding that is supported by Graziadio et al. (2015), who only observed increased CLT in older adults in a task that is known to cause unilateral activation in younger but *bilateral* activation in older adults.

In summary, based on the current results it seems that ageing is not associated with a change in the efficacy of MVF. Protocols that combine MVF with unilateral motor training might therefore be as efficient in modulating bilateral neurophysiological and behavioural changes as in younger adults, with some people benefiting more from that special type of intervention than others. Considering the assumed association between neurophysiological and behavioural changes in the untrained limb is still poorly understood, together with the substantial degree of inter-participant variability expressed in the current projects, it is suggested that future MVF-related research in older adults should focus on further investigating the behavioural benefits from a clinical/functional perspective rather than solely investigating mechanistic neuroplastic adaptations.

6.3. Future Directions

The findings from this thesis suggest that provision of MVF within unilateral motor paradigms does not confer additional ipsilateral CSE increases or additional performance gains in the untrained limb over those observed when more standard forms of visual feedback (i.e., focus on the active or inactive limb) are provided. This conclusion contrasts with recent work from other labs (Avanzino et al., 2014; Lappchen et al., 2012; Nojima et al., 2012; Zult et al., 2015), and indicates that if MT is to be adapted more widely in an extended rehabilitation setting, further research demonstrating consistent and robust findings (across different tasks, different cohorts of participants of sufficient sample size and, ultimately, reproduced across multiple different research groups) are required.

In an attempt to boost the behavioural benefits of MVF, recent work has extended traditional MT by incorporating NIBS techniques, such as transcranial direct current stimulation (tDCS). NIBS can be applied before or during the training paradigm on the basis that neural plasticity induced via brain stimulation will interact with the plasticity induced by way of undertaking the motor task (i.e., use-dependent plasticity). The theory is that the overall plastic response in the untrained hemisphere is accentuated by NIBS, which will result in greater behavioural gains in the ipsilateral untrained limb. Promising results using such combined intervention protocols have been reported within the context of both simple (Jax, Rosa-Leyra, & Coslett, 2015) and complex motor tasks (Hoff et al., 2015; von Rein et al., 2015). Upregulating the hemisphere corresponding to the limb behind the mirror via anodal tDCS, augmented the effect of MVF by solidifying the well-known dominance of vision over proprioception (Jax et al., 2015). As a consequence, when asked to

perform a simple reaching task with the hand hidden behind the mirror, participants were more biased by the mirror image of the stationary hand positioned offset in front of the mirror, and thus less likely to correctly estimate the exact position of the hand positioned behind the mirror. Furthermore, work from a different laboratory showed anodal tDCS to result in enhanced CLT in a complex motor task compared to conditions that solely employed MVF or anodal tDCS (Hoff et al., 2015; von Rein et al., 2015) in both younger and older adults. These recent works suggest that combined NIBS/MVF approaches are worth pursuing in order to improve the overall outcomes of rehabilitation programmes.

NIBS techniques might also be used in a different context to up- or downregulate areas outside the M1 to investigate how MVF affects neural mechanisms connecting secondary and preparatory motor regions and M1. On the basis of the results reported in chapters 3 and 5, it can be hypothesised that MVF-driven changes do not occur within the M1 and that net excitability (as investigated by traditional TMS measurements) might not be the driving factor underlying the beneficial effect of mirror feedback. Rather, it is possible that MVF-related changes occur upstream of M1, or within the parietal or occipital lobe, and that those changes might vary over the lifespan. Future studies investigating / modulating such networks might therefore be helpful to uncover the neural mechanisms underlying the behavioural effects of MT.

On a different note, future studies assessing the degree of kinaesthetic illusion elicited through MVF would be very beneficial in determining the underpinnings of the illusory technique. It is well known that the provision of MVF is able to create cognitive (i.e., a mismatch between expected and actual feedback) and perceptual

(i.e., a mismatch between visual and kinaesthetic feedback) conflicts (Deconinck et al., 2015; Metral et al., 2014; Romano, Bottini, & Maravita, 2013). In a recent study, Nojima et al. (2015) demonstrated that kinaesthetic illusions are an important component for motor learning and M1 plasticity induced by action observation. The more participants felt that an observed video clip of a third person performing a motor task elicited an illusory sensation in the corresponding limb, the more they improved when subsequently performing the observed task with the same limb. As the effects induced by observing a mirror image of a moving limb have been associated with those elicited by action observation interventions, future work should incorporate questionnaires asking participants about the vividness of the MVF-induced illusion in their hand hidden behind the mirror. Ensuring that a strong illusory effect is present seems to be important to determine the success of MVF-based interventions.

In light of the fact that all studies in the present thesis were performed in healthy and high functioning younger and older participants, the question still remains to what degree those results can be generalized to clinical populations. I therefore encourage, despite the “negative” results, more research to be conducted within a clinical setting, as MVF might affect neural plasticity differently in specific, neurologically impaired populations compared to healthy individuals. Considering the potential effect of MVF on multiple functional networks (for an overview see Deconinck et al., 2015), MT might activate different mechanisms depending on the specific neurological damage that exists in the participants undertaking MVF-combined training interventions. Regardless of whether MT-induced neural plasticity and performance improvements are related, MVF might serve as a stimulus to augment functional recovery in areas of the impaired hemisphere responsible for

limb control (Howatson et al., 2013; Saleh, Adamovich, & Tunik, 2014), and thereby lead to more pronounced behavioural effects in the corresponding limb. In light of studies showing that MVF-protocols neither specifically modulate bimanual coordination (Metral et al., 2014; Selles et al., 2014) nor outweigh the effects of interventions performed with the impaired hand, it can be suggested that future research should especially focus on those people with more severe neurological impairments where the severity of the condition precludes, or vastly restricts, use of the impaired or damaged limb.

6.4. Concluding remarks

This work reported in this thesis addressed the important question of how MVF can specifically modulate bilateral neurophysiological and behavioural changes when provided during unilateral training across the lifespan. This issue has significant implications if MT is to be fully exploited as a potential treatment or intervention tool in the ageing population, a fact that is highlighted by the recent increase in papers investigating MVF and CLT in young and older populations since the commencement of the present thesis (e.g., Avanzino et al., 2014; Dickins et al., 2015; Hoff et al., 2015; Howatson et al., 2013; Nojima et al., 2015; von Rein et al., 2015; Zult et al., 2015). In the tasks employed in the current thesis, it was reported that MVF did not lead to enhanced CSE or CLT in either younger or older adults. MVF-induced behavioural changes were determined to most likely occur due to a combination of mechanisms relating to transfer of a motor action, together with action observation resulting from attending to a particular type of visual feedback. While young and older adults exhibited similar degrees of CLT, this was associated with different age-specific parameters, suggesting shifts in the mechanisms underlying the transfer. The present thesis has advanced the understanding of MT, and in doing so has opened new possibilities for related research in the future.

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APPENDICES

Study 1

PARTICIPANT INFORMATION SHEET

The role of visual feedback in corticospinal excitability

Invitation: You are invited to participate in a study investigating how visual feedback alters cortical excitability when undertaking simple movements with one hand. The aim is to determine how visual feedback influences cortical excitability and how this role of visual feedback changes across the lifespan spectrum. This research is funded by a Discovery Early Career Research Award [DE120100729].

The study is being conducted by:

- Paola Reissig, School of Psychology, University of Tasmania

The study will be conducted in the Human Motor Control Laboratory, Psychology Research Centre, University of Tasmania, (03) 6226 2558. The study consists of a single session which will last approximately 2 hours.

Study Procedures

The following procedures will be used in this research: (a) recording of muscle activity (EMG), (b) transcranial magnetic stimulation (TMS), (c) motor task.

(a) **EMG:** EMG is a technique to record the electrical activity of muscles both in response to TMS - see (b) - and during your movements – see (c). At the beginning of the experiment, small, self-adhesive recording electrodes will be affixed to the skin over the muscle of interest.

Wires will be connected to the electrodes to allow the muscle activity to be recorded by a computer. To ensure the best possible recording, the skin will be prepared by scrubbing it with a mildly abrasive paste and then cleaning it with an alcohol wipe. If there is excessive hair on the skin (e.g., forearm muscles) a small area may be shaved using a disposable razor. This procedure may produce some minor irritation of the skin (e.g., redness). The adhesives used on the electrodes are hypoallergenic.

(b) **Non-invasive Brain Stimulation (NIBS):** During the experiment, activity of the brain areas involved in movement will be measured using a technique called Transcranial Magnetic Stimulation (TMS). TMS is a safe, painless and commonly used technique to study brain activity. It is used extensively by investigators in the Human Motor Control Laboratory. Electromagnetic 'pulses' will be delivered through one or two coils held against your scalp by the investigator. To ensure the coil/s is always positioned in the same place, a felt-tip pen will be used to mark the location/s on your scalp. This mark will be removed at the end of the session using an alcohol wipe. When the pulse is delivered you will hear an audible 'click' and muscles of the hand/arm will 'twitch'. You may also feel a 'tap' sensation on your scalp and muscles around the eye may twitch, causing the eye to blink. This may feel a bit strange but it is not painful.

Experimental procedure: During the testing sessions you will be seated in an adjustable chair. Recording electrodes will be placed over the relevant muscles. At the beginning of the experiment, the scalp location and TMS intensities will be determined. This will involve moving the TMS coil to different scalp positions and applying TMS of varying intensities. This part of the experiment will take approximately 30 minutes and will provide baseline measures of the level of cortical excitability.

(c) **Motor task:** In the main part of the experiment, you will be asked to perform a motor task, which involves a simple, unilateral index finger abduction (and adduction) movement towards a marked spot on the table. An auditory metronome will be used to pace the movement. One complete abduction-adduction cycle will be performed on each beat of the metronome. You are asked to synchronise the finger abduction with the onset of the metronome beat. You will be asked to move your finger towards the marked spot on the table and to cover that spot at the point of the maximal finger movement (use it as reversal point). It is important that you execute the task using moderate force (approximately 20% maximum), so that you will be able to maintain the movement throughout the whole experiment. While you are performing the task, TMS will be delivered occasionally after the onset of the metronome.

You will receive familiarisation trials prior to the main experiment in which there will be 3 different conditions. In two of these conditions, the motor task will be performed under different viewing conditions: Active (Mirror), and No Vision. In the Active Vision condition, you will visually fixate your active hand while vision of the unattended hand will be prevented by covering it with a wooden box. In the No Vision condition, you will be looking straight ahead and vision of both hands will be prevented by covering them with two boxes. In the Mirror Vision condition, you will watch a mirror reflection of your active hand, with your inactive hand positioned (unseen) behind the mirror such that the reflected hand will appear superimposed over top of it. For each block of trials in each of the conditions you will be required to make 60 movements, once every second (1 min per block). Additionally,

there will be a Baseline condition in which both hands are relaxed and only TMS will be applied intermittently.

The Baseline and the No Vision condition will be conducted three times with the right hand making the movements, and three times with the left hand. The Active (Mirror) Vision condition will be conducted 6 times. As such there will be 24 blocks of trials in total, each block taking 2 minute.

You will be allowed rest breaks between blocks and are free to request a rest break at any time during the session. As such, this part of the experiment will take approximately 2 hours.

Inclusion and Exclusion criteria

Individuals (male and female) between 18 and 35 years and between 60 and 80 years of age are invited to participate in this research. Interested volunteers should have normal or corrected-to-normal vision, and have no known neuromuscular or neurological disorders, or recent injuries of the hands or arms.

TMS is a very safe technique; however there are certain conditions that will exclude some people from participating. These include:

- epilepsy, or a family history of epilepsy
- history of unexplained seizures (fits)
- serious head injury (e.g., concussion) requiring hospitalisation within the last three years
- implanted electronic devices such as pacemakers
- metal implants or metal fragments in the head (excluding dental work)
- history of migraines
- pregnancy

Please ask the experimenter if you are unsure of any of these.

Certain medications (for example some types of anti-depressant medications) can influence how the brain responds to sensory stimulation and voluntary movements. ***Therefore, we ask that you inform the experimenter if you are taking any medication prior to participating in the study***.

Risks & Discomforts:.

There are few possible risks or discomforts associated with these procedures: however some individuals may experience mild discomfort while receiving transcranial magnetic stimulation. The TMS pulse may cause muscles of the scalp to 'twitch' (e.g., can cause the eye to blink). This may feel 'odd', but is not painful. On rare occasions TMS can cause a 'muscle tension' type headache. If at any time you feel you have a headache, please let the experimenter know immediately. The electrodes that record muscle activity of TMS responses may cause some mild skin irritation and redness. You may experience some minor muscle fatigue as a result of performing voluntary movements. If your muscles become uncomfortable as a result of the movements, please inform the experimenter. In

general, if at any time you feel uncomfortable for any reason, please inform the experimenter and the procedures can immediately be stopped.

Remuneration:

For remuneration your name will either receive 15 Dollars or course credit for the total time you were involved in the experiment. The investigators will be available after the session to answer any questions you may have regarding the investigation.

Confidentiality:

Your individual experimental data will be coded alpha-numerically and stored on a secure computer server that will be available only to the investigators via a password system. All future use of your data will be by the alpha-numeric code only to ensure anonymity. Your data will be retained securely at the University of Tasmania for at least five years. When it is no longer required by law, your data will be destroyed by the deletion of electronic files and shredding of documents

Voluntary participation:

Participation in the study is completely voluntary. If you agree to participate, you are free to withdraw from the study at any time without prejudice. If participation is for course-credit and you withdraw, you will receive credit for the time you have participated, up to a maximum of 2 hours. Otherwise, you will receive $5 for each half-hour of participation, up to a maximum of $20. If you withdraw from the study, any data that you have supplied can be identified through the alpha-numeric coding system and withdrawn from the study if you wish. You will be asked to sign an informed consent form to evidence your consent to participate in the study. Consent forms will be locked in a filing cabinet in the Cognitive and Motor Aging Laboratory at the University of Tasmania and kept separately from your data.

Contact persons: If you wish to obtain more information, please contact the following researcher:

Ms Paola Reissig (6226 2558 or Paola.Reissig@utas.edu.au)

This study has been approved by the Tasmanian Social Sciences Human Research Ethics Committee. If you have concerns or complaints about the conduct of this study, please contact the Executive Officer of the HREC (Tasmania) Network on (03) 6226 7479 or email human.ethics@utas.edu.au. The Executive Officer is the person nominated to receive complaints from research participants. Please quote ethics reference number **H0012891.** You will be provided with a copy of this information sheet and a statement of informed consent to keep. It can be expected that results of individual studies will be available within a year of data collection.

You will be provided with a copy of this information sheet and a statement of informed consent to keep.

Date...../...../..... Participant Code..... Age..... yrs..... mths Sex: M /

Medical and History Questionnaire[1]

Medical History

Are you currently suffering from anxiety or depression?.....

Do you have a heart condition or any other serious physical condition?

.....

Are you currently taking any prescription medication? If so, what medication?

.....

Have in the past taken any medications for psychological condition(s)? If so, what medications?

.....

Have you ever had or are you now suffering from any of the following (please circle):

Stroke	Yes	No
High Blood Pressure > 140 / 90	Yes	No
Diabetes	Yes	No
Arthritis	Yes	No
Fits or convulsions	Yes	No
Epilepsy	Yes	No

Giddiness	Yes	No
Concussion	Yes	No
Severe Head Injury	Yes	No
Loss of Consciousness	Yes	No

Handedness

For each of the activities below, please tell us:

Which hand do you prefer for that activity?

Do you *ever* use the other hand for the activity?

Preferred hand?	Ever use other hand?			
Writing	L	R	Y	N
Drawing	L	R	Y	N
Throwing	L	R	Y	N
Using scissors	L	R	Y	N
Using a toothbrush	L	R	Y	N
Using a knife (without fork)	L	R	Y	N
Using a spoon	L	R	Y	N
Using a broom (upper hand)	L	R	Y	N
Striking a match	L	R	Y	N
Opening a box (lid)	L	R	Y	N

Do you ever confuse left and right?……..

How many people in your immediate family are left handed?……

Drinking and Smoking History (please circle 1 answer)

On how many days last week did you drink alcohol?	None
	One or two days
	Three or four days
	Five or six days
	Every day
Do you usually drink..	Never
	Occasionally
	During weekdays
	Friday night
	Weekends
How many drinks would you usually have at one time?	One or two
	Three to five
	Five to eight
	Eight to twelve
	More than twelve
Do you get drunk?	Never
	Rarely
	Once a month
	Once a week
	More frequently
How often do you smoke a cigarette?	Never
	Less than 5 per week

Less than 5 per day

5 to 9 per day

10 to 19 per day

20 to 39 per day

Over 40 per day

Do you or have you in the past used marijuana? (please circle) Yes No

a) Have you used marijuana in the last two weeks? Yes No

b) Have you used any other form of illicit drug in the last 6 months? Yes No

Vision

Do you have any difficulties with vision? (please specify)

.....

If yes, are these difficulties corrected (i.e. glasses/contacts)

.....

Note: It is a formal requirement of the Human Research Ethics Committee (Tasmania) Network that the information provided on this questionnaire be held under security to comply with confidentiality regulations and to protect your privacy. You can be assured that information will be available only to the principal researcher and not to any other party. The questionnaire will be destroyed following completion of the project.

Thank you for your Participation!

School of Psychology

Informed Consent Form

1. I have read and understood the Information Sheet for this study.

2. I understand that this experimental session will be lasting approximately 2 hours.

3. I understand that transcranial magnetic stimulation *may* cause a little discomfort during stimulus delivery to the scalp.

4. I do not have a cardiac pacemaker, metal implants, or medical pumps in my body. I do not have any metal in my head such as shrapnel, surgical clips or fragments from welding. I do not suffer from seizures and there is no history of seizures in the members of my immediate family. I have not had neurosurgery and I have not had a head injury severe enough to require hospitalisation. I do not suffer from frequent or severe headaches. I do not have haemophilia.

5. I understand that as remuneration I will either receive 15 Dollars or I will receive course credit for the total time I am involved with the experiment.

6. I understand that all research data will be securely stored on the University of Tasmania premises for a period of 5 years. Electronic data will be stored on a password protected computer. All data will be destroyed at the end of 5 years.

7. Any questions that I have asked have been answered to my satisfaction.

8. I agree that research data gathered for the study may be published provided that I cannot be identified as a subject.

9. I agree to participate in this investigation and understand that I may withdraw at any time without any effect. Following completion of the experiment, please contact a researcher if you wish to have your data withdrawn from the study for any reason. Data can be withdrawn at any

time until submission of the manuscripts for publication (~ 6-12 months following completion of data collection).

Name of Participant: _____

Signature of Participant: _____Date:_____

I have explained this project and the implications of participation in it to this participant, and I believe that the consent is informed and that he/she understands the implications of participation.

Name of Investigator: _____

Signature of Investigator: _____Date:_____

Study 2

PARTICIPANT INFORMATION SHEET

The role of visual feedback on training-induced performance gains

Invitation: You are invited to participate in a study investigating how visual feedback alters performance gains after a unilateral motor task with your dominant hand. This research is funded by a Discovery Early Career Research Award [DE120100729].

The study is being conducted by:

- Paola Reissig, School of Psychology, University of Tasmania

The study will be conducted in the Human Motor Control Laboratory, Psychology Research Centre, University of Tasmania, (03) 6226 2558. The study consists of a single session, which will last approximately 20-30 minutes.

Motor task:

You will be seated in a height adjustable chair with your forearms rested on a table and their palms facing upwards. You will then be asked to rotate two golf balls as quickly as possible in either a clockwise direction (with your right hand) or an anti-clockwise direction (with your left hand).

During the main part of the experiment you will be provided with different types of feedback. You will either be asked to focus on your active or your inactive hand, or on the mirror reflection of your active hand.

You will participate 10 blocks of 30 seconds of ball rotation. Thirty seconds of rest will be provided between each practice block to avoid fatigue. Prior to, and following the training phase (total duration 10 min), you will perform the same task with your non-dominant hand for 30 seconds with similar instructions (i.e., to perform the task as quickly as possible).

Data of the first and last training block of the trained hand and the two test blocks of the untrained hand was collected via video recordings and stored for subsequent analysis.

Inclusion and Exclusion criteria:

Individuals (male and female) between 18 and 50 years are invited to participate in this research. Interested volunteers should have normal or corrected-to-normal vision, and have no known neuromuscular or neurological disorders, or recent injuries of the hands or arms.

Remuneration:

Your research participation is on a voluntary basis and you will not receive any reimbursement other than course credit for the total time that you are involved in the experiment.

Confidentiality:

Your individual experimental data will be coded alpha-numerically and stored on a secure computer server that will be available only to the investigators via a password system. All future use of your data will be by the alpha-numeric code only to ensure anonymity. Your data will be retained securely at the University of Tasmania for at least five years. When it is no longer required by law, your data will be destroyed by the deletion of electronic files and shredding of documents. Consent forms will be locked in a filing cabinet in the Cognitive and Motor Aging Laboratory at the University of Tasmania and kept separately from your data.

Contact persons: If you wish to obtain more information, please contact the following researcher:

Ms Paola Reissig (6226 2558 or Paola.Reissig@utas.edu.au)

This study has been approved by the Tasmanian Social Sciences Human Research Ethics Committee. If you have concerns or complaints about the conduct of this study, please contact the Executive Officer of the HREC (Tasmania) Network on (03) 6226 7479 or email human.ethics@utas.edu.au. The Executive Officer is the person nominated to receive complaints from research participants. Please quote ethics reference number **H0012891.** You will be provided with a copy of this information sheet and a statement of informed consent to keep. It can be expected that results of individual studies will be available within a year of data collection.

You will be provided with a copy of this information sheet and a statement of informed consent to keep.

Date...../...../..... Participant Code..... Age..... yrs..... mths Sex: M /

Handedness Questionnaire

Handedness

For each of the activities below, please tell us:

Which hand do you prefer for that activity?

Do you *ever* use the other hand for the activity?

Preferred hand?	Ever use other hand?			
Writing	L	R	Y	N
Drawing	L	R	Y	N
Throwing	L	R	Y	N
Using scissors	L	R	Y	N
Using a toothbrush	L	R	Y	N
Using a knife (without fork)	L	R	Y	N
Using a spoon	L	R	Y	N
Using a broom (upper hand)	L	R	Y	N
Striking a match	L	R	Y	N
Opening a box (lid)	L	R	Y	N

Do you ever confuse left and right?……..

How many people in your immediate family are left handed?……

Thank you for your Participation!

School of Psychology

Informed Consent Form

1. I have read and understood the Information Sheet for this study.
2. I understand that this experimental session will be lasting approximately 20-30 minutes.
3. I understand that my research participation is on a voluntary basis and that I will not receive any reimbursement other than course credit for the total time I am involved with the experiment.
4. I understand that all research data will be securely stored on the University of Tasmania premises for a period of 5 years. Electronic data will be stored on a password protected computer. All data will be destroyed at the end of 5 years.
5. Any questions that I have asked have been answered to my satisfaction.
6. I agree that research data gathered for the study may be published provided that I cannot be identified as a subject.
7. I agree to participate in this investigation and understand that I may withdraw at any time without any effect. Following completion of the experiment, please contact a researcher if you wish to have your data withdrawn from the study for any reason. Data can be withdrawn at any time until submission of the manuscripts for publication (~ 6-12 months following completion of data collection).

Name of Participant: _____

Signature of Participant: _____Date:_____

I have explained this project and the implications of participation in it to this participant, and I believe that the consent is informed and that he/she understands the implications of participation.

Name of Investigator: _____

Signature of Investigator: _____Date:_____

Study 3

PARTICIPANT INFORMATION SHEET

The role of visual feedback in corticospinal excitability and performance gains

Invitation: You are invited to participate in a study investigating age-related changes in the ability of the brain to learn and perform motor tasks. The aim of this research is to improve our understanding of how the two hemispheres of the brain interact when people undertake simple unimanual movements and how this communication might change over age. This research is funded by a Discovery Early Career Research Award [DE120100729].

The study is being conducted by:

- Paola Reissig, School of Psychology, University of Tasmania

The study will be conducted in the Human Motor Control Laboratory, Psychology Research Centre, University of Tasmania, (03) 6226 2558. The study consists of a single session which will last approximately 2.5 hours.

Study Procedures

The following procedures will be used in this research: (a) recording of muscle activity (EMG), (b) transcranial magnetic stimulation (TMS), (c) motor task.

(a) EMG: EMG is a technique to record the electrical activity of muscles both in response to TMS - see (b) - and during your movements – see (c). At the beginning of the experiment, small, self-adhesive recording electrodes will be affixed to the skin over the muscle of interest. Wires will be connected to the electrodes to allow the muscle activity to be recorded by a computer. To ensure the best possible recording, the skin will be prepared by scrubbing it with a mildly abrasive paste and then cleaning it with an alcohol wipe. If there is excessive hair on the skin (e.g., forearm muscles) a small area may be shaved using a

disposable razor. This procedure may produce some minor irritation of the skin (e.g., redness). The adhesives used on the electrodes are hypoallergenic.

(b) **Non-invasive Brain Stimulation (NIBS):** During the experiment, activity of the brain areas involved in movement will be measured using a technique called Transcranial Magnetic Stimulation (TMS). TMS is a safe, painless and commonly used technique to study brain activity. It is used extensively by investigators in the Human Motor Control Laboratory. Electromagnetic 'pulses' will be delivered through one or two coils held against your scalp by the investigator. To ensure the coil/s is always positioned in the same place, a felt-tip pen will be used to mark the location/s on your scalp. This mark will be removed at the end of the session using an alcohol wipe. When the pulse is delivered you will hear an audible 'click' and muscles of the hand/arm will 'twitch'. You may also feel a 'tap' sensation on your scalp and muscles around the eye may twitch, causing the eye to blink. This may feel a bit strange but it is not painful.

Experimental procedure: During the testing sessions you will be seated in an adjustable chair. Recording electrodes will be placed over the relevant muscles. In addition, two accelerometers will be attached to both index fingers to collect behavioural measurements.

At the beginning of the experiment, the scalp location and TMS intensities will be determined. This will involve moving the TMS coil to different scalp positions and applying TMS of varying intensities. This part of the experiment will take approximately 30 minutes and will provide baseline measures of the level of cortical excitability.

(c) **Motor task:** In the main part of the experiment, you will be asked to perform a unilateral ballistic abduction movement with your left and right index finger, while keeping the rest of the hand still. The aim of the task will be to maximise the horizontal peak acceleration of each movement, measured using an accelerometer attached to the index finger with a plastic splint and tape.

In the main part of the experiment you will perform two blocks of 150 trials of the same task with your dominant hand and will be provided with one of two forms of feedback during performance. In the Active Vision group you will be asked to focus on your active hand, while in the Mirror Vision a mirror you will be asked to focus on the mirror reflection of your active hand. You will receive auditory feedback in the form of a high or a low pitch tone after each trial, informing you whether peak acceleration on the preceding trial has been better (high tone) or worse (low tone) than the previous trial. You will be familiarised with both tones before the start of the experiment to ensure your ability to distinguish them.

A rest period of 30 seconds will be given every 15 trials, therefore dividing the training period into 20 sub-blocks. We will collect neurophysiological (i.e., TMS data) and behavioural data (i.e., acceleration data – 10 trial per hand) bilaterally before and after each training block (i.e., mid-test and post-test respectively). As such, this part of the experiment will take approximately 2 hours.

Inclusion and Exclusion criteria

Individuals (male and female) between 18 and 35 years and between 60 and 80 years of age are invited to participate in this research. Interested volunteers should have normal or corrected-to-normal vision, and have no known neuromuscular or neurological disorders, or recent injuries of the hands or arms.

TMS is a very safe technique; however there are certain conditions that will exclude some people from participating. These include:

- epilepsy, or a family history of epilepsy
- history of unexplained seizures (fits)
- serious head injury (e.g., concussion) requiring hospitalisation within the last three years
- implanted electronic devices such as pacemakers
- metal implants or metal fragments in the head (excluding dental work)
- history of migraines
- pregnancy

Please ask the experimenter if you are unsure of any of these.

Certain medications (for example some types of anti-depressant medications) can influence how the brain responds to sensory stimulation and voluntary movements. ***Therefore, we ask that you inform the experimenter if you are taking any medication prior to participating in the study***.

Risks & Discomforts:

There are few possible risks or discomforts associated with these procedures: however some individuals may experience mild discomfort while receiving transcranial magnetic stimulation. The TMS pulse may cause muscles of the scalp to 'twitch' (e.g., can cause the eye to blink). This may feel 'odd', but is not painful. On rare occasions TMS can cause a 'muscle tension' type headache. If at any time you feel you have a headache, please let the experimenter know immediately. The electrodes that record muscle activity of TMS responses may cause some mild skin irritation and redness. You may experience some minor muscle fatigue as a result of performing voluntary movements. If your muscles become uncomfortable as a result of the movements, please inform the experimenter. In general, if at any time you feel uncomfortable for any reason, please inform the experimenter and the procedures can immediately be stopped.

Remuneration:

For remuneration your name will either receive 15 Dollars or course credit for the total time you were involved in the experiment. The investigators will be available after the session to answer any questions you may have regarding the investigation.

Confidentiality:

Your individual experimental data will be coded alpha-numerically and stored on a secure computer server that will be available only to the investigators via a password system. All future use of your data will be by the alpha-numeric code only to ensure anonymity. Your data will be retained securely at the University of Tasmania for at least five years. When it is no longer required by law, your data will be destroyed by the deletion of electronic files and shredding of documents

Voluntary participation:

Participation in the study is completely voluntary. If you agree to participate, you are free to withdraw from the study at any time without prejudice. If participation is for course-credit and you withdraw, you will receive credit for the time you have participated, up to a maximum of 2 hours. Otherwise, you will receive $5 for each half-hour of participation, up to a maximum of $20. If you withdraw from the study, any data that you have supplied can be identified through the alpha-numeric coding system and withdrawn from the study if you wish. You will be asked to sign an informed consent form to evidence your consent to participate in the study. Consent forms will be locked in a filing cabinet in the Cognitive and Motor Aging Laboratory at the University of Tasmania and kept separately from your data.

Contact persons: If you wish to obtain more information, please contact the following researcher:

Ms Paola Reissig (6226 2558 or Paola.Reissig@utas.edu.au)

This study has been approved by the Tasmanian Social Sciences Human Research Ethics Committee. If you have concerns or complaints about the conduct of this study, please contact the Executive Officer of the HREC (Tasmania) Network on (03) 6226 7479 or email human.ethics@utas.edu.au. The Executive Officer is the person nominated to receive complaints from research participants. Please quote ethics reference number **H0012891.** You will be provided with a copy of this information sheet and a statement of informed consent to keep. It can be expected that results of individual studies will be available within a year of data collection.

You will be provided with a copy of this information sheet and a statement of informed consent to keep.

TMS PRE-SESSION SCREENING FORM

Name: ……

Age: ……..

Sex: M / F / Unspecified

Do you have any difficulties with vision? (please detail) ……....

If so, are these difficulties corrected, and how? ……

Are you currently taking any medication? (please detail) ……...

Before receiving TMS, please read the following questions carefully and provide answers. For a small number of individuals, TMS may carry an increased risk of causing a seizure. The purpose of these questions is to make sure that you are not such a person. You have the right to withdraw from the screening and subsequent session if you find the questions unacceptably intrusive. The information you provide will be treated as strictly confidential and will be held in secure conditions. If you are unsure of the answer to any of the questions, please ask the person who gave you this form or the person who will be performing the study.

Have you ever had an adverse reaction to TMS?	**Y / N**
Do you have a heart condition?	**Y / N**
Do you or does anyone in your family have epilepsy?	**Y / N**
Have you or anyone in your family ever had a seizure?	**Y / N**
Have you ever had neurosurgery or a serious head injury requiring hospitalisation?	**Y / N**
Have you ever had a stroke?	**Y / N**
Do you have any metal in your head (outside the mouth) such as shrapnel, surgical clips, or fragments from welding or metalwork?	**Y / N**
Do you have any implanted devices such as cardiac pacemakers, aneurysm clips, cochlear implants, shunt, stent?	**Y / N**

Have you ever had any other brain related condition, or illness that has caused brain injury?	**Y / N**
Are you taking or have you in the past taken any psychiatric or neuroactive medications (e.g. antidepressants)?	**Y / N**
Are you pregnant or could you possibly be pregnant?	**Y / N**
Have you ever been told that your blood pressure is specifically high or low?	**Y / N**
Do you have diabetes?	**Y / N**
Do you have arthritis?	**Y / N**
Do you or have you ever suffered from giddiness?	**Y / N**
Have you ever experienced loss of consciousness (i.e. syncope or fainting)?	**Y / N**
Have you ever had a concussion?	**Y / N**
Do you suffer from migraines, or frequent/severe headaches?	**Y / N**
Do you have haemophilia (a disorder impairing the body's ability to control blood clotting/coagulation)?	**Y / N**
Have you ever undergone electroconvulsive therapy (ECT)?	**Y / N**
Do you have any hearing problems or ringing in your ears?	**Y / N**

If you answered 'yes' to any of the above questions or have any other serious physical condition, please provide details below:

.....
.....
.....
.....
.....
.....

Handedness Inventory

For each of the activities below, please tell us:

1. Which hand do you prefer for that activity?
2. Do you *ever* use the other hand for the activity?

	Preferred hand?		Ever use other hand?	
Writing	L	R	Y	N
Drawing	L	R	Y	N
Throwing	L	R	Y	N
Using scissors	L	R	Y	N
Using a toothbrush	L	R	Y	N
Using a knife (without fork)	L	R	Y	N
Using a spoon	L	R	Y	N
Using a broom (upper hand)	L	R	Y	N
Striking a match	L	R	Y	N
Opening a box (lid)	L	R	Y	N

Do you ever confuse left and right? ……..

How many people in your immediate family are left handed? ……

IMMEDIATE HISTORY

To minimise the risk of TMS causing an adverse effect, it is important that you answer the following questions accurately before we begin the session.

In the last 12 hours, have you consumed more than 3 units of alcohol?	Y / N
In the last 12 hours, have you consumed any recreational drugs?	Y / N
Did you get a good night's sleep last night, and do you feel alert?	Y / N
In the last two hours, have you consumed more than 2 cups of coffee, or any other caffeinated drinks?	Y / N
Would you like to be provided with any further information regarding TMS?	Y / N

I have read and understood the questions above and have answered them correctly.

Signed…….. Date……

In the presence of …… (Name) ……(Signature)

Note: It is a formal requirement of the Human Research Ethics Committee (Tasmania) Network that the information provided on this questionnaire be held securely to comply with confidentiality regulations and to protect your privacy. You can be assured that information will be available only to the principal researcher and not to any other party. The questionnaire will be destroyed following completion of the project.

THANK YOU FOR YOUR PARTICIPATION!

School of Psychology

Informed Consent Form

1. I have read and understood the Information Sheet for this study.
2. I understand that this experimental session will be lasting approximately 2.5 hours.
3. I understand that transcranial magnetic stimulation *may* cause a little discomfort during stimulus delivery to the scalp.
4. I do not have a cardiac pacemaker, metal implants, or medical pumps in my body. I do not have any metal in my head such as shrapnel, surgical clips or fragments from welding. I do not suffer from seizures and there is no history of seizures in the members of my immediate family. I have not had neurosurgery and I have not had a head injury severe enough to require hospitalisation. I do not suffer from frequent or severe headaches. I do not have haemophilia.
5. I understand that as remuneration I will either receive 20 Dollars or I will receive course credit for the total time I am involved with the experiment.
6. I understand that all research data will be securely stored on the University of Tasmania premises for a period of 5 years. Electronic data will be stored on a password protected computer. All data will be destroyed at the end of 5 years.
7. Any questions that I have asked have been answered to my satisfaction.
8. I agree that research data gathered for the study may be published provided that I cannot be identified as a subject.
9. I agree to participate in this investigation and understand that I may withdraw at any time without any effect. Following completion of the experiment, please contact a researcher if you wish to have your data withdrawn from the study for any reason. Data can be withdrawn at any time until submission of the manuscripts for publication (~ 6-12 months following completion of data collection).

Name of Participant: _____

Signature of Participant: _____Date:_____

I have explained this project and the implications of participation in it to this participant, and I believe that the consent is informed and that he/she understands the implications of participation.

Name of Investigator: _____

Signature of Investigator: _____Date:_____